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The Journal of Clinical Investigation

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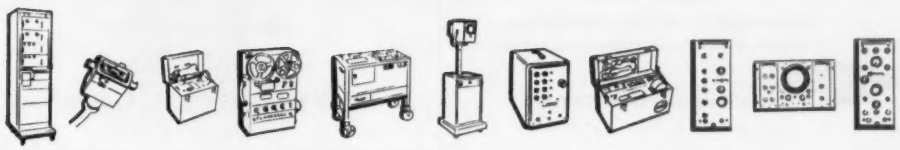

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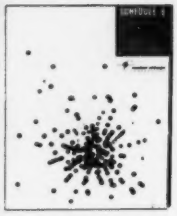

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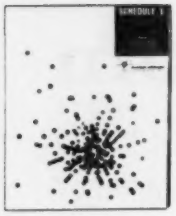

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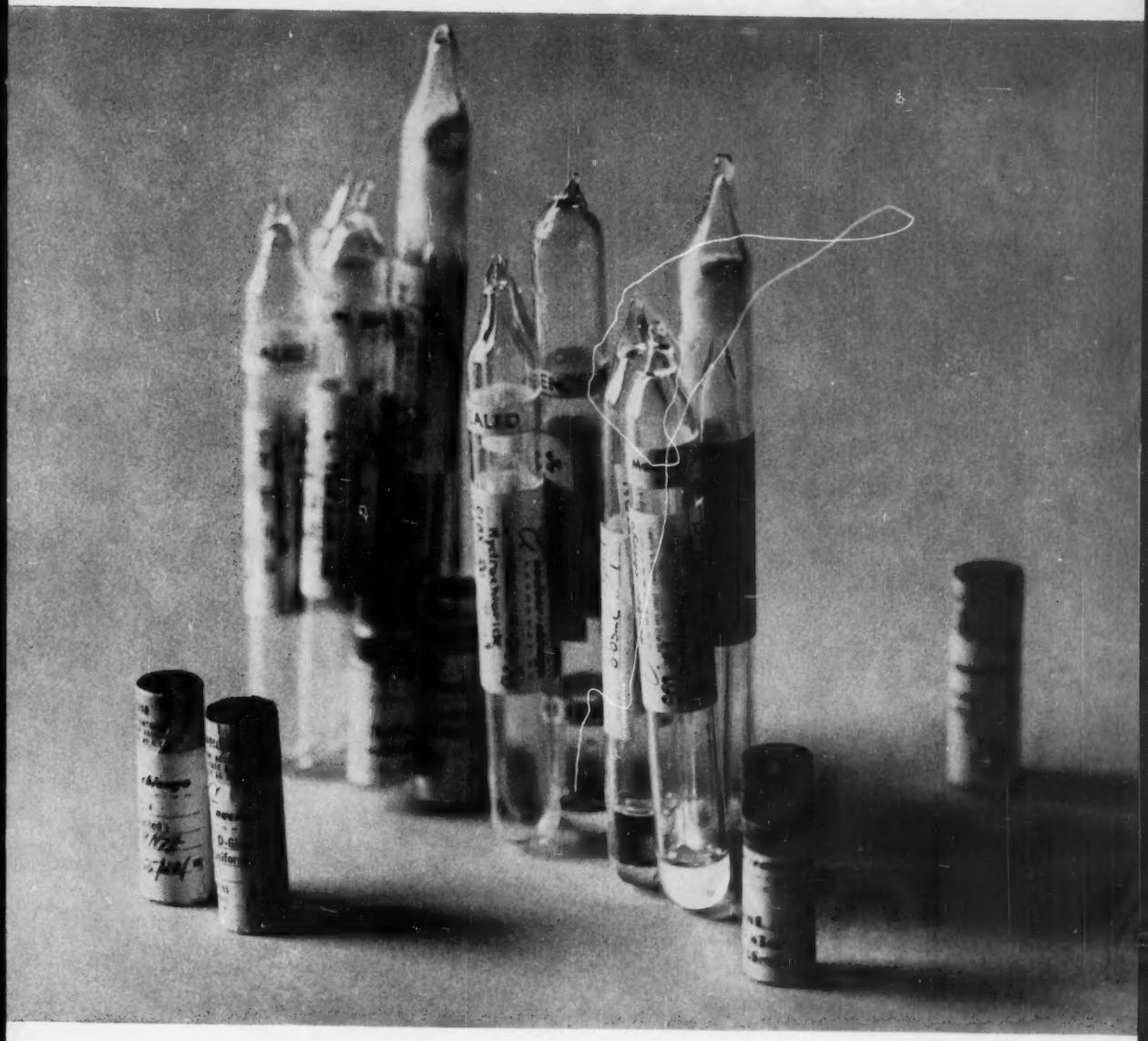

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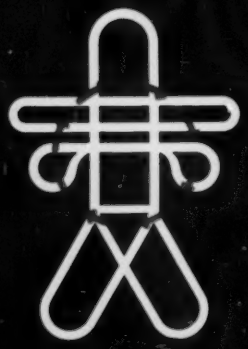

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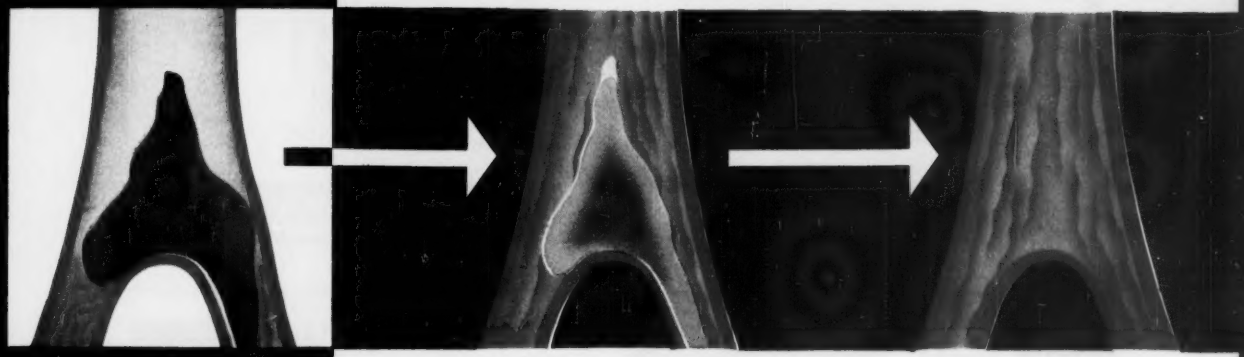

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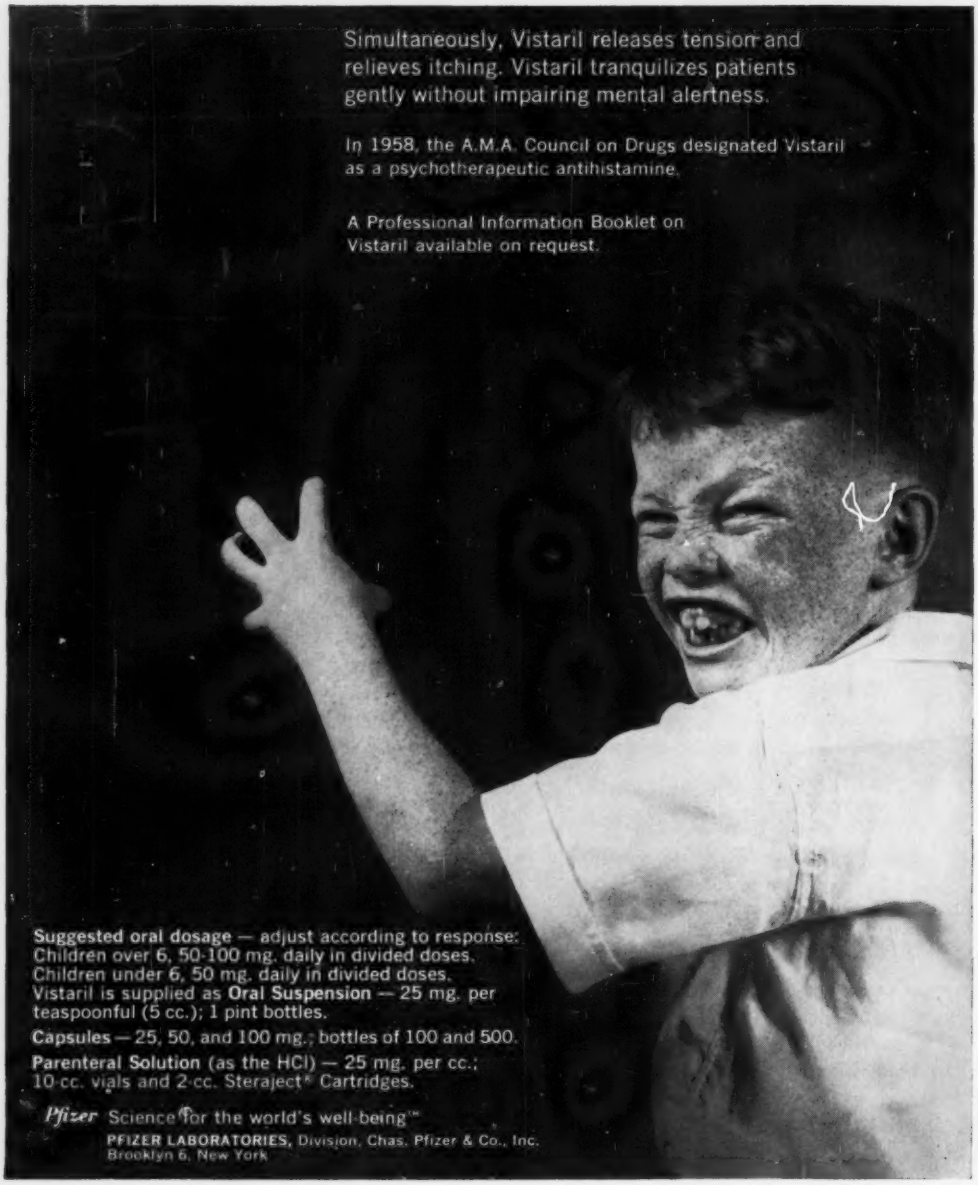

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CHARACTERISTICS OF RENAL BICARBONATE REABSORPTION IN MAN *

By DONALD W. SELDIN, RICHARD M. PORTWOOD,† ‡ FLOYD C. RECTOR, JR. AND ROBERT CADE §

(From The Department of Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Tex.)

(Submitted for publication January 26, 1959; accepted June 5, 1959)

The reabsorption of filtered HCO_3^- by the kidney is thought to result not from the active reabsorption of HCO_3^- ions but rather from the secretion of cellular H^+ in exchange for tubular Na^+ (1, 2). The secreted H^+ reacts with filtered HCO_3^- to form H_2CO_3 which then decomposes to CO_2 and H_2O, thereby effecting the reabsorption of $NaHCO_3$. The enzyme carbonic anhydrase, the inhibition of which blocks HCO_3^- reabsorption, is thought to maintain an adequate supply of H^+ for secretion by accelerating the hydration of CO_2 within the renal cells. In support of this theory is the fact that HCO_3^- reabsorption varies linearly with plasma pCO_2 (3–5).

Recently Schwartz, Falbriard and Relman (6) have suggested an alternative role for carbonic anhydrase. By a kinetic analysis of the effects of partial inhibition of carbonic anhydrase on HCO_3^- reabsorption during severe metabolic acidosis they found that at a given dose of Diamox® the reciprocals of HCO_3^- reabsorption and of plasma HCO_3^- concentration were linearly related. As carbonic anhydrase was progressively inhibited with increasing doses of Diamox® a family of lines typical of substrate-enzyme-inhibitor kinetics was obtained. From these relationships it was suggested that HCO_3^- was reabsorbed by some mechanism in which cellular carbonic anhydrase was the enzyme and filtered HCO_3^- (or some intermediate derived from it) was the substrate for the enzyme.

In order to examine further the role of carbonic anhydrase in the reabsorption of HCO_3^-, the effect of Diamox® on the relationship between HCO_3^- reabsorption and plasma pCO_2 was studied in normal subjects and in subjects with preexisting metabolic acidosis. During the course of these experiments, certain relations between HCO_3^- reabsorption, plasma HCO_3^- concentration and plasma pCO_2 became apparent which suggested that CO_2[1] rather than filtered HCO_3^- constituted the substrate involved in HCO_3^- reabsorption. A series of *in vitro* experiments was then performed in which the kinetics obtained by Schwartz and his associates (6) were reduplicated, although neither enzyme nor inhibitor was involved.

MATERIAL AND METHODS

A total of 24 experiments was performed on 13 normal young men. All studies were done in the morning, the subjects having fasted overnight. The subjects remained recumbent throughout except when voiding. Maximum water diuresis was maintained in all experiments by the intravenous infusion of 5 per cent fructose (in some cases glucose) in water following the oral ingestion of 1,500 to 2,000 ml. of distilled water.

In five types of experiments the effects of carbonic anhydrase inhibition alone and in combination with respiratory alkalosis, respiratory acidosis, metabolic acidosis, and mixed metabolic acidosis-respiratory alkalosis were observed. Carbonic anhydrase inhibition was produced by a single intravenous injection of 250 mg. of Diamox®. The alterations in acid-base composition were induced as follows:

1) Respiratory alkalosis—by voluntary hyperventilation, assisted by a Halliburton Intermittent Positive Pressure Breathing (IPPB) machine delivering 100 per cent oxygen.

2) Respiratory acidosis—by inhalation of 6 to 6.5 per cent CO_2 in oxygen.

3) Metabolic acidosis—by oral ingestion of a total of 20 to 25 Gm. of NH_4Cl during the 24 hours preceding the

* This work was supported in part by a grant from the National Institutes of Health, United States Public Health Service, and in part by a grant from the Dallas Heart Association.

† Work done as a Public Health Service Trainee of the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

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§ Present address: The Department of Physiology, Cornell University Medical College, New York, N. Y.

[1] In this paper it is assumed that plasma and cellular CO_2 tensions are identical; therefore plasma pCO_2 reflects the effective concentration of intracellular CO_2.

TABLE I

The effect of Diamox® on bicarbonate reabsorption during acute changes in plasma acid-base composition

Subject, Age, Ht., Wt., Experiment	Time	Plasma			Urine							
		pH	pCO_2	HCO_3^-	Flow	C_{In}	pH	pCO_2	HCO_3^- filt.	HCO_3^- exc.	HCO_3^- reabsorbed	
	min.		*mm. Hg*	*mEq./ L.*	*ml./ min.*	*ml./ min.*		*mm. Hg*	*μEq./ min.*	*μEq./ min.*	*μEq./ min.*	*mEq./ 100 ml. G. filtrate*
N. K.	0–20	7.38	41	23.4	26.0		5.94	68		31		
26 yr.	20–40*	7.38	40	23.0	22.5	123	6.02	73	2,829	34	2,795	2.27
1.8 M.	40–60	7.38	40	22.9	30.5	130	6.85	134	2,977	567	2,410	1.85
85 Kg.	60–80	7.37	41	22.8	21.0	108	7.00	121	2,462	496	1,966	1.82
	80–100	7.36	41	22.3	22.0	114	6.92	122	2,542	436	2,106	1.85
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N. K.	0–21	7.37	35	19.7	17.9	163	5.67	51	3,211	8	3,203	1.96
26 yr.	21–41*	7.35	37	19.5	18.5	142	5.65	56	2,769	8	2,761	1.94
1.8 M.	41–62†	7.34	38	20.0	26.9		7.11	78		468		
85 Kg.	62–82	7.54	19	15.9	31.0	126	7.19	60	2,003	499	1,504	1.19
	82–102	7.55	17	14.4	26.2	110	7.21	48	1,584	354	1,230	1.12
Respiratory	102–122†	7.56	17	14.3	24.0	113	7.09	57	1,616	293	1,323	1.17
alkalosis +	122–142	7.41	30	18.1	19.8	95	6.98	65	1,720	216	1,504	1.58
Diamox®	142–162	7.36	34	18.8	21.0	123	6.91	73	2,312	218	2,094	1.70
N. K.	0–15	7.38	41	23.5	27.3		6.38	52		60		
27 yr.	15–30*	7.39	40	23.6	22.3	159	6.27	59	3,752	47	3,705	2.33
1.8 M.	30–45‡	7.39	40	23.7	28.0	151	7.16	65	3,579	512	3,067	2.03
85 Kg.	45–60	7.27	56	24.8	27.0	131	7.20	83	3,249	697	2,552	1.95
Respiratory	60–75‡	7.23	59	24.0	32.0	159	7.07	94	3,816	688	3,128	1.97
acidosis +	75–90	7.34	41	21.5	21.3	130	7.16	76	2,795	456	2,339	1.80
Diamox®	90–105	7.41	37	22.6	12.7	129	7.26	94	2,915	425	2,490	1.93
C. P.	0–20	7.24	42	17.4	37.5	209	5.83	43	3,820	22	3,798	1.81
26 yr.	20–40*	7.23	41	16.5	31.5	167	5.87	44	2,895	19	2,876	1.72
1.8 M.	40–60	7.23	42	17.1	41.0	172	6.63	86	3,090	295	2,795	1.63
80 Kg.	60–80	7.23	42	17.0	37.5	153	6.86	52	2,730	277	2,453	1.60
Metabolic	80–100	7.25	40	16.7	36.5	155	6.8[illegible]		2,720	223	2,497	1.67
acidosis +	100–120	7.24	42	17.3	33.5	144	6.		2,620	161	2,459	1.71
Diamox®	120–140	7.27	37	16.5	34.5	166	6.[illegible]	42	2,880	141	2,739	1.65
T. L.	0–20	7.29	37	17.1	19.0	147	5.63	46	2,514	8	2,506	1.70
27 yr.	20–40*	7.32	35	17.3	19.0	150	5.64	42	2,595	8	2,587	1.72
1.8 M.	40–60†	7.30	38	17.9	26.8	141	6.93	62	2,524	276	2,248	1.59
78 Kg.	60–80	7.41	23	14.3	25.8	129	7.00	54	1,845	279	1,566	1.21
NH_4Cl +	80–100	7.50	16	12.1	25.0	126	7.06	44	1,525	250	1,275	1.01
respiratory	100–115†	7.55	13	11.4	22.0	125	7.03	44	1,425	200	1,225	0.98
alkalosis + Diamox®	115–135	7.29	34	15.9	22.0	138	6.92	59	2,194	209	1,985	1.44

* Injection of 250 mg. Diamox® intravenously.
† Period of hyperventilation.
‡ Period of breathing 6 per cent CO_2.

experiment. In several studies chronic administration of 15 Gm. NH_4Cl daily for one to two weeks preceded the acute load.

4) Mixed metabolic acidosis-respiratory alkalosis—by hyperventilation in subjects who had previously ingested the 20 to 25 Gm. of NH_4Cl.

The analytical methods were those described in a previous paper (*7*).

RESULTS

The results are presented in protocol form in Table I with inclusion of a representative example of each of the several types of experiments. Since chronic NH_4Cl acidosis was associated with the same response as acute NH_4Cl loads, only one instance of the latter is charted. It is apparent from these data that, regardless of plasma acid-base composition, the administration of Diamox® was associated with a reduction in HCO_3^- reabsorption whether expressed in absolute terms or as mEq. reabsorbed per 100 ml. of glomerular filtrate.

In Figure 1 the effects of the different experimental procedures on plasma [HCO_3^-] and pCO_2 are plotted. It is noteworthy that metabolic acido-

sis was accompanied by only a slight decrease in plasma pCO_2 despite a marked depression in plasma concentration of HCO_3^-. In the other experimental states the plasma $[HCO_3^-]$ was related to pCO_2 in a roughly linear fashion.

Following the administration of Diamox®, HCO_3^- reabsorption was linearly related to plasma pCO_2 (Figure 2). The regression equation describing this relationship is $Y = 0.61 + 0.028$ X. Brazeau and Gilman (3), Relman, Etsten and Schwartz (4) and Dorman, Sullivan and Pitts (5) have previously shown that HCO_3^- reabsorption was linearly related to plasma pCO_2 in the presence of normal carbonic anhydrase activity. Thus, inhibition of carbonic anhydrase does not disturb this linear relationship.

The administration of Diamox® during NH_4Cl acidosis resulted in moderate HCO_3^- excretion (200 to 300 μEq. per minute) despite the reduced concentration of HCO_3^- in plasma. As seen in Figure 2 the values for HCO_3^- reabsorption are somewhat skewed below the regression line. Although this skewed distribution is not striking, it is conceivable that the reduced concentration of HCO_3^- in glomerular filtrate during NH_4Cl acidosis partially limited the reabsorption of HCO_3^- despite the excretion of moderate amounts of HCO_3^- into the urine.

Schwartz and associates (6) have reported that at a given dose of Diamox® HCO_3^- reabsorption was approximately proportional to plasma HCO_3^- concentration. In the present experiments this same proportionality was also apparent (Figure 3). However, at approximately the same plasma $[HCO_3^-]$, the reabsorption of HCO_3^- was much less during respiratory alkalosis than during metabolic acidosis (Figure 3). In respiratory alkalosis and metabolic acidosis, plasma $[HCO_3^-]$ (and the filtered load of HCO_3^-) were comparably depressed, yet during respiratory alkalosis 30 to 40 per cent of the filtered HCO_3^- was excreted while in metabolic acidosis only 7 to 15 per cent of the filtered HCO_3^- was excreted. This difference in HCO_3^- reabsorption between respiratory alkalosis and metabolic acidosis was probably the consequence of the fact that in respiratory alkalosis plasma pCO_2 falls more than $[HCO_3^-]$ does, whereas in metabolic acidosis the depression in plasma $[HCO_3^-]$ is far greater than any decrease in plasma pCO_2 (Figure 1). These observations suggest that during HCO_3^- diuresis plasma pCO_2 is a more important determinant of HCO_3^- reabsorption than is the concentration of HCO_3^-.

To examine whether the correlation between plasma $[HCO_3^-]$ and HCO_3^- reabsorption in a reciprocal plot establishes the existence of an interaction between filtered HCO_3^- and cellular carbonic anhydrase in the course of HCO_3^- reab-

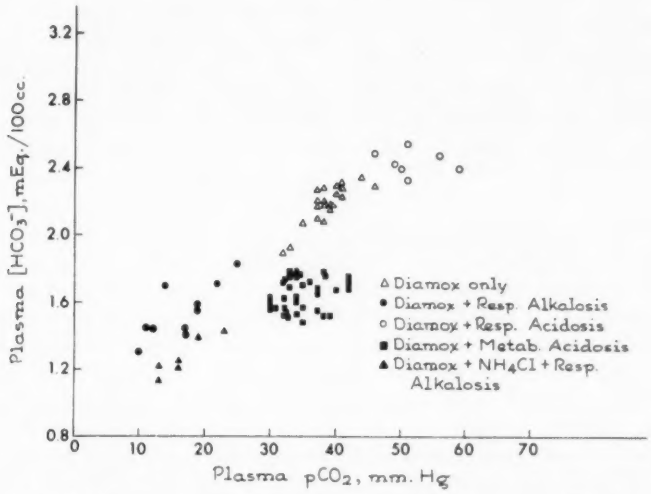

FIG. 1. EFFECT OF ACUTE CHANGES IN ACID-BASE BALANCE ON PLASMA pCO_2 AND HCO_3^- CONCENTRATION

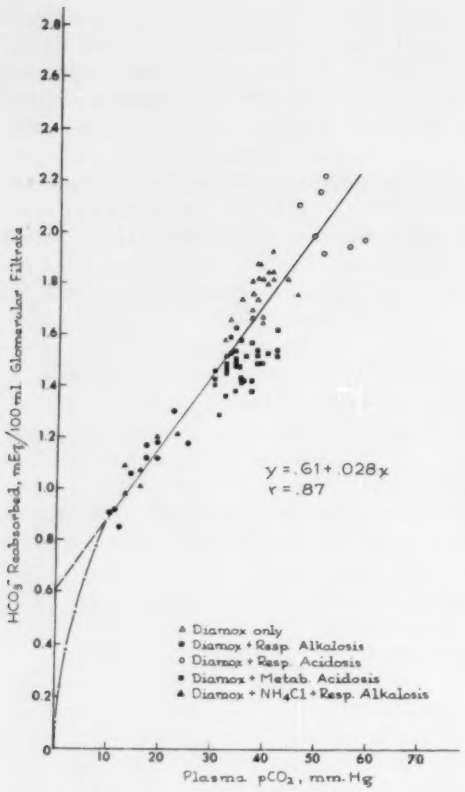
HCO_3^- Reabsorbed, mEq/100 ml Glomerular Filtrate
Plasma pCO_2, mm. Hg
y = .61 + .028x
r = .87
Diamox only
Diamox + Resp. Alkalosis
Diamox + Resp. Acidosis
Diamox + Metab. Acidosis
Diamox + NH_4Cl + Resp. Alkalosis

FIG. 2. RELATION OF HCO_3^- REABSORPTION TO PLASMA pCO_2 FOLLOWING DIAMOX® ADMINISTRATION DURING ACUTE CHANGES IN ACID-BASE BALANCE

The plasma pCO_2 could not be reduced below 14 mm. Hg because of the development of tetany. For this reason the configuration of the lower portion of the curve could not be examined. The dotted extensions to the vertical axis represent two alternative possibilities. This area of the curve is currently being studied in dogs.

sorption, as suggested by Schwartz and colleagues (6), or whether this same relationship might also be the result of H^+ secretion, *in vitro* studies (Table II) were performed to simulate the effects of secretion of H^+ ions into bicarbonate-containing tubular fluid. Fifty ml. aliquots of seven different HCO_3^- solutions, each containing 15 mMoles per L. sodium phosphate (pH 7.4), were placed in 100 ml. fritted glass funnels and aerated with 8.23 per cent CO_2 for 15 minutes. At the end of this period 3 ml. samples were aspirated into oiled syringes for measurement of the initial [HCO_3^-] which ranged from 93.96 to 8.97 mEq. per L. Next, 0.1 ml. 2.5 N HCl was added to each funnel. Again the samples were aerated for 15 minutes with 8.23 per cent CO_2 and 3 ml. aliquots were once more removed for HCO_3^- determinations. An additional 0.1 ml. 2.5 N HCl (giving a cumulative value of 0.50 mEq. HCl) was then added to each funnel and the procedure repeated. Altogether a total of 0.4 ml. of 2.5 N HCl was added to each funnel in increments of 0.1 ml. The difference between the initial HCO_3^- concentration and the HCO_3^- concentration after each increment of acid represents the quantity of HCO_3^- dissipated by the addition of HCl.

It is clear from Table II that the quantity of HCO_3^- decomposed is not equivalent to the quantity of acid added, but more nearly approximates it at the higher initial HCO_3^- concentrations. The data in Table II are plotted in Figure 4, where the initial [HCO_3^-] concentrations are plotted along the horizontal axis and the quantities of HCO_3^- decomposed (mEq. per L.) by each addition of acid are plotted along the vertical axis. It is apparent from this figure that the quantity of HCO_3^- decomposed for each quantity of acid added is related to the initial [HCO_3^-] in a curvilinear fashion. If the reciprocal of the decomposed HCO_3^- (1/V) is plotted against the reciprocal of the initial HCO_3^- concentration (1/S), a family of four straight lines is obtained, one for each total quantity of acid added (Figure 5). These lines intercept at the same point on the 1/S axis in a fashion typical of lines obtained by the kinetic analysis of noncompetitive enzyme inhibition, despite the fact that neither enzyme nor inhibitor participated in the *in vitro* reactions. The results of a similar experiment in which the concentration of sodium phosphate was 5 mMoles per L. rather than 15 mMoles per L. are plotted reciprocally in Figure 6. The lines are similar to those in Figure 5. A comparison of Figures 5 and 6 reveals that decreasing the buffer concentration decreases the slope of the lines. The results of these *in vitro* studies are in accord with what have been predicted from the kinetics of buffer equilibria; *i.e.*, the addition of HCl to buffered bicarbonate solutions can yield kinetics characteristic of noncompetitive enzyme inhibition.

These *in vitro* reactions are analogous to the reabsorption of HCO_3^- by the secretion of H^+ into

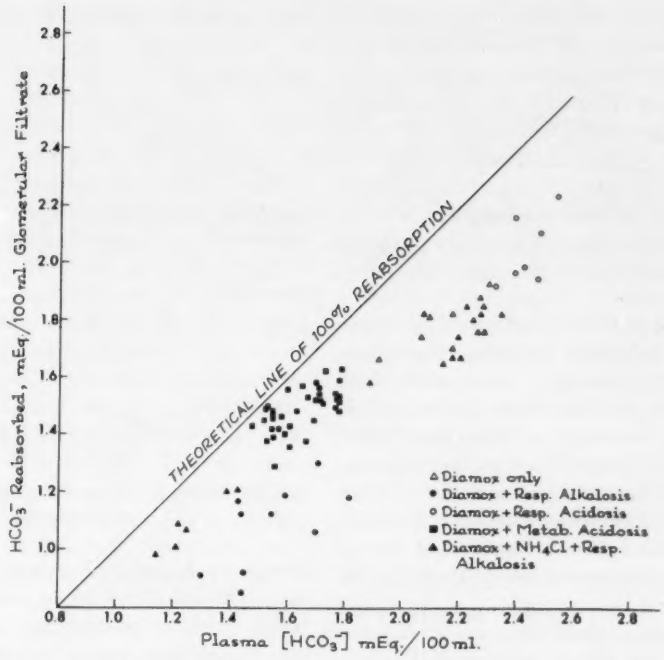
HCO_3^- Reabsorbed, mEq./100 ml. Glomerular Filtrate
Plasma $[HCO_3^-]$ mEq./100 ml.
THEORETICAL LINE OF 100% REABSORPTION
Diamox only
Diamox + Resp. Alkalosis
Diamox + Resp. Acidosis
Diamox + Metab. Acidosis
Diamox + NH_4Cl + Resp. Alkalosis

FIG. 3. RELATION OF BICARBONATE REABSORPTION TO PLASMA HCO_3^- FOLLOWING DIAMOX® ADMINISTRATION DURING ACUTE ALTERATIONS IN ACID-BASE BALANCE

the tubular lumen. It should not be inferred, however, that the *in vitro* model exactly duplicated the conditions existing in the nephron; for instance, the concentration of phosphate buffer used in these studies greatly exceeded the concentration of phosphate usually present in glomerular filtrate. The studies simply demonstrated that the reciprocal relationship between plasma HCO_3^- concentration and HCO_3^- reabsorption during the administration of Diamox® could arise from competition between bicarbonate and nonbicarbonate buffer systems for secreted H^+. The competing buffering action, however, did not necessarily originate in the glomerular filtrate, but could, instead, have arisen as

TABLE II

The addition of HCl to phosphate-buffered bicarbonate solutions

Initial $[HCO_3^-]$*†	47 ml. HCO_3^- sol. + 0.25 mEq. HCl (5.32 mEq./L.)		44 ml. HCO_3^- sol. + 0.50 mEq. HCl (11.38 mEq./L.)		41 ml. HCO_3^- sol. + 0.75 mEq. HCl (18.30 mEq./L.)		38 ml. HCO_3^- sol. + 1.00 mEq. HCl (26.30 mEq./L.)	
	$[HCO_3^-]$*	HCO_3^- decomposed	$[HCO_3^-]$*	HCO_3^- decomposed	$[HCO_3^-]$*	HCO_3^- decomposed	$[HCO_3^-]$*	HCO_3^- decomposed
mEq./L.	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*
93.96	89.75	4.21	82.74	11.22	77.07	16.89	70.71	23.25
71.63	67.58	4.05	60.75	10.88	55.60	16.03	49.28	22.35
47.99	44.12	3.87	37.67	10.32	32.84	15.15	26.94	21.05
29.09	25.68	3.41	20.27	8.82	15.50	13.59	10.34	18.75
20.12	17.12	3.00	12.05	8.07	7.87	12.25	3.60	16.52
12.07	9.60	2.47	5.65	6.42	2.34	9.73		
8.97	6.88	2.08	3.43	5.53				

* After equilibration with 8.23 per cent CO_2.
† All solutions contained 15 mMoles per L. sodium phosphate pH 7.4.

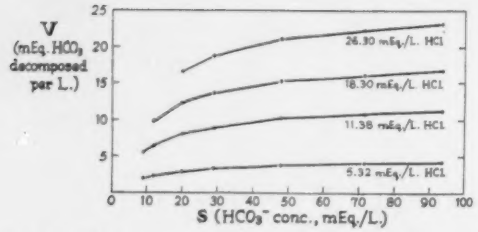

FIG. 4. TITRATION OF PHOSPHATE-BUFFERED BICARBONATE SOLUTIONS WITH HYDROCHLORIC ACID

a result of cellular processes. If increasing inhibition of carbonic anhydrase by Diamox® progressively diminished the available supply of H^+ ions and at the same time rendered the mechanism for net H^+ secretion sensitive to intraluminal pH,[2] then filtered HCO_3^- would, in effect, be competing with cellular processes for secreted H^+. Under these conditions progressive elevation of the concentration of filtered HCO_3^- could yield curves similar to those shown in Figure 5. Thus, similar kinetics would be obtained regardless of whether Diamox® diminished the secretion of H^+ into the tubular fluid or decreased the number of enzyme sites available for interaction with filtered HCO_3^-.

[2] Net secretion of H^+ during carbonic anhydrase inhibition could be influenced by intraluminal pH in several ways. Davies (8) has recently suggested that carbonic anhydrase is located on the luminal surface of the tubular cell; carbonic anhydrase inhibition could result in accumulation of H_2CO_3, thereby accelerating the back-diffusion of secreted H^+. Inhibition of carbonic anhydrase could also increase the back-diffusion of H^+ by increasing the permeability of the cell membrane to H^+ ions. Finally, inhibition of carbonic anhydrase could sensitize the mechanism for active H^+ transport to small changes in the pH of tubular urine.

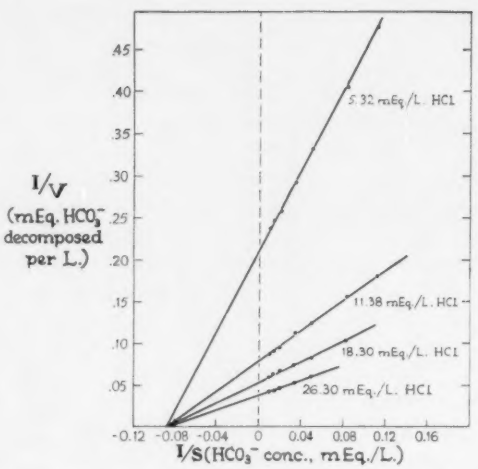

FIG. 5. RECIPROCAL PLOT OF BICARBONATE TITRATION EXPERIMENT

DISCUSSION

The reabsorption of filtered HCO_3^- by the kidney in many respects resembles an active transport process involving enzyme-carrier molecules. Most of the criteria usually invoked to establish the existence of a specific carrier mecha-

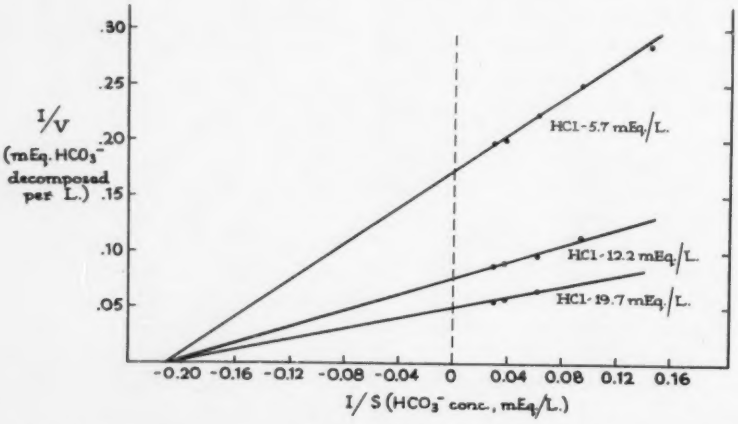

FIG. 6. RELATIONSHIP BETWEEN BICARBONATE CONCENTRATION AND DECOMPOSITION OF BICARBONATE BY ADDITION OF HYDROCHLORIC ACID

nism, *i.e.* saturation, competitive inhibition, specific inhibition, and kinetics characteristic of an enzyme-substrate interaction (9), have been demonstrated for the reabsorption of HCO_3^-. First, Pitts and Lotspeich (10) have shown that in normal animals elevation of the filtered load of HCO_3^- saturated the tubular reabsorptive mechanism at a HCO_3^- Tm of approximately 2.6 mEq. per 100 ml. glomerular filtrate. Second, Hilton and his associates (11) have presented evidence that in acute respiratory acidosis the administration of NaCl depressed the HCO_3^- Tm, suggesting competition between Cl^- and HCO_3^- for carrier sites. Third, carbonic anhydrase inhibitors specifically block HCO_3^- reabsorption without impairing other metabolic processes of renal tissue. Finally, Schwartz and co-workers (6) have found that the relationship between HCO_3^- reabsorption and the concentration of plasma HCO_3^- during partial inhibition of carbonic anhydrase with Diamox® was typical of substrate-enzyme-inhibitor kinetics. In the light of these data, the possibility exists, at least on theoretical grounds, that the formation of a bicarbonate-carbonic anhydrase complex mediates the active removal of HCO_3^- from the tubular lumen.

According to a more widely accepted theory (1, 2), the primary process in HCO_3^- reabsorption is the secretion of cellular H^+ in exchange for tubular Na^+. The secreted H^+ reacts with HCO_3^- to form H_2CO_3 which then decomposes to CO_2 and H_2O. According to this theory the H^+ involved in HCO_3^- reabsorption arises from the hydration of CO_2 catalyzed by carbonic anhydrase.

Two of the criteria listed as evidence supporting the theory of active HCO_3^- reabsorption, *i.e.* saturation and specific inhibition with Diamox®, however, are equally consistent with the theory of H^+ secretion. So, too, the enhanced excretion of HCO_3^- following NaCl loads (11) is not conclusive evidence of competitive inhibition, inasmuch as this effect could equally well result from a limitation on Na^+ reabsorption. The question then arises of whether the demonstration of typical substrate-enzyme-inhibtor kinetics (6) establishes the existence of active HCO_3^- reabsorption involving carbonic anhydrase as the carrier molecule, or whether these same kinetics are compatible with the theory that HCO_3^- is reabsorbed as the passive consequence of H^+ secretion. The results of our *in vitro* experiments showed that a linear relationship between the reciprocals of HCO_3^- reabsorption and plasma $[HCO_3^-]$ was nonspecific, equally compatible with either of the above mentioned theories of HCO_3^- reabsorption.

While the results of these *in vitro* studies do not exclude the existence of active HCO_3^- reabsorption involving carbonic anhydrase as the carrier molecule, other lines of evidence render it unlikely. First, the elevated urinary CO_2 tensions observed during $NaHCO_3$ infusions (10) could not be explained if active transport were the sole mechanism for HCO_3^- reabsorption. Second, the linear relationship between plasma pCO_2 and HCO_3^- reabsorption (3–5) would be difficult to explain if the reabsorption of HCO_3^- were accomplished solely by an interaction between cellular carbonic anhydrase and filtered HCO_3^-. To explain this effect it would be necessary to propose that the activity of carbonic anhydrase varied linearly with pCO_2. Studies in this laboratory by Carter, Seldin and Teng (12), however, have shown that the activity of carbonic anhydrase in rat kidney was not measurably increased in chronic respiratory acidosis. While it is possible that CO_2 increases the *in vivo* activity of the enzyme in such a way that no change is detectable by an *in vitro* assay, Roughton and Booth (13) have demonstrated that lowering pH actually depressed the activity of carbonic anhydrase. Thus, intracellular acidosis accompanying respiratory acidosis would be expected to inhibit rather than accelerate HCO_3^- reabsorption. The linear relation between plasma pCO_2 and HCO_3^- reabsorption, therefore, supports the theory that HCO_3^- is reabsorbed as the passive consequence of H^+ secretion rather than the active removal of HCO_3^- ions from the tubule lumen.

The failure of complete carbonic anhydrase inhibition to abolish HCO_3^- reabsorption (14) raises the question of whether there might be a second mechanism for the reabsorption of HCO_3^- not involving the secretion of H^+. The results of the present studies, however, indicate that following the administration of 250 mg. Diamox® intravenously HCO_3^- reabsorption is still linearly related to plasma pCO_2, despite marked inhibition of carbonic anhydrase activity.[3] This suggests that

[3] Counihan, Evans and Milne (15) have shown that 90 to 95 per cent of the maximally achievable inhibitory

all HCO_3^- reabsorption results from the secretion of H^+ and that the uncatalyzed hydration of CO_2 is an important source of H^+ for the Na^+–H^+ exchange process.

The demonstration that the uncatalyzed hydration of CO_2 constitutes an important source of H^+ for the reabsorption of HCO_3^- affords an explanation for the failure of Diamox® to produce maximum HCO_3^- diuresis during metabolic acidosis. It was shown in Figure 1 that the concentration of plasma HCO_3^- was depressed in NH_4Cl acidosis without a commensurate decrease in plasma pCO_2. Therefore, under these circumstances, the quantity of HCO_3^- filtered was reduced, but the capacity of the uncatalyzed hydration of CO_2 to effect the reabsorption of HCO_3^- remained comparatively unchanged. Thus, when the plasma HCO_3^- concentration fell in metabolic acidosis to the point where the filtration of HCO_3^- was equal to the capacity of the uncatalyzed hydration of CO_2 to reabsorb HCO_3^-, Diamox® no longer produced a HCO_3^- diuresis. Following the administration of 250 mg. Diamox® the reabsorption of HCO_3^- at a plasma pCO_2 of 35 mm. Hg (the average pCO_2 during metabolic acidosis) was approximately 1.5 mEq. per 100 ml. glomerular filtration rate (GFR) (Figure 2), presumably due to the uncatalyzed hydration of CO_2 and any residual uninhibited carbonic anhydrase. Therefore, in man given this amount of Diamox® reabsorption of HCO_3^- would be virtually complete when the plasma HCO_3^- concentration fell below about 15 mEq. per L., a level similar to that obtained in man by others (15). On the other hand, depression of the plasma HCO_3^- concentration to 15 mEq. per L. by hyperventilation drastically diminished the capacity of the uncatalyzed hydration of CO_2 to reabsorb HCO_3^-, and hence Diamox® would be expected to produce marked HCO_3^- diuresis despite the smaller quantity of filtered HCO_3^-.

SUMMARY

The pattern of renal HCO_3^- reabsorption during various alterations in acid-base balance was examined in 13 normal subjects with and without the administration of Diamox®. Following the administration of Diamox® HCO_3^- reabsorption varied linearly with plasma pCO_2, as described by the regression equation $Y = 0.61 + 0.028X$. This linear relationship in the presence of marked inhibition of carbonic anhydrase indicated that the uncatalyzed hydration of CO_2 was an important source of H^+ for the reabsorption of HCO_3^-. The failure of Diamox® to produce marked HCO_3^- diuresis during metabolic acidosis was attributable to nearly complete reabsorption of the small filtered load via the uncatalyzed hydration of CO_2.

In vitro studies, in which varying amounts of HCl were added to phosphate-buffered HCO_3^- solutions, disclosed that the linear relationship between the reciprocals of HCO_3^- reabsorption and plasma HCO_3^-, used as evidence for the active reabsorption of HCO_3^-, was equally compatible with the theory that HCO_3^- is reabsorbed as the passive consequence of the secretion of H^+.

effects of Diamox® are obtained in man by the intravenous injection of 250 mg. of the drug.

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CORRECTION

On page 1279 of the paper "Evidence that a humoral agent stimulates the adrenal cortex to secrete aldosterone in experimental secondary hyperaldosteronism" by N. A. Yankopoulos, J. O. Davis, B. Kliman and R. E. Peterson (J. clin. Invest. 1959, **38**, 1278), credit for the double isotope derivative method is incorrectly attributed. The double isotope derivative procedure for analysis of aldosterone was developed by Kliman and Peterson. An editorial error resulted in the insertion of Davis and Yankopoulos in the citation.

OBSERVATIONS ON CERULOPLASMIN IN WILSON'S DISEASE *†

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(Submitted for publication January 29, 1959; accepted June 19, 1959)

In the light of present knowledge hepatolenticular degeneration is classified as a metabolic disease, probably based on an inborn error of metabolism and transmitted by an autosomal recessive gene. The biochemical manifestations of Wilson's disease have been studied extensively and were summarized in several recent reviews (1–4). The findings point to aberrations in copper and protein metabolism, but the nature of the basic metabolic defect and the mechanism of the disease are still in the realm of speculation.

The most consistent biochemical abnormality in Wilson's disease is the deficiency of the copper containing α_2 globulin, ceruloplasmin. It was suggested that a defect in the production of this protein may be the basic metabolic abnormality, and Wilson's disease has been classed accordingly as one of the pathological conditions resulting from the congenital deficiency of a specific plasma protein (5, 6).

This paper describes, for the first time as far as we are aware, two patients with Wilson's disease and normal serum concentration of ceruloplasmin. These two patients were investigated in detail, including studies with radioactive copper, in order to substantiate their diagnosis and also in the hope that the results might contribute to our knowledge of the basic mechanism of Wilson's disease. For the purpose of comparison a third patient who exhibited all the typical clinical and biochemical characteristics of Wilson's disease was also studied by the same methods.

CASE MATERIAL

Rob. M. and Ron. M. were brothers, sons of second cousins. The older (Rob. M.) had onset of symptoms at 10 years of age and was diagnosed and treated by us for some time as a case of posthepatitic cirrhosis. Two years later his younger brother (Ron. M.) presented at 11 years of age, with hepatosplenomegaly, an indurated liver and slight but definite laboratory evidence of liver dysfunction. This prompted investigation of the sibs for familial causes of cirrhosis. In the course of these studies both were found to have an increased urinary copper excretion and aminoaciduria. Kayser-Fleischer rings were detected in both by slit-lamp examination. Neither of the patients developed neurological signs and symptoms during life. They both ran a progressively downhill course as far as their liver disease was concerned, with bouts of jaundice, and gradual loss of activity and appetite, followed by the development of portal hypertension and intractable ascites. Both died in hepatic coma approximately four years after the onset of signs and symptoms of their disease.

The third patient (Mar. Ch.) exhibited all the characteristic clinical and biochemical manifestations of hepatolenticular degeneration. She was admitted at 12 years of age for the investigation of neurological symptoms suggestive of extrapyramidal tract damage. She had evidence of cirrhosis, biochemical findings typical of Wilson's disease and, in addition, Kayser-Fleischer rings. Her neurological symptoms improved following prolonged treatment with BAL (2,3-dimercaptopropanol) and penicillamine (β,β-dimethyl cysteine); her cirrhosis however progressed relentlessly. She developed portal hypertension and died in hepatic coma following portacaval anastomosis.[1]

METHODS

The method of Eden and Green was used to measure copper in the serum and quantitative 24 hour collections of urine (7). For tissue copper determinations 1 to 30 Gm. of fresh tissue was digested completely in redistilled concentrated nitric acid (with a trace of Antifoam® [2]). The resulting clear solutions were concentrated by further boiling, transferred quantitatively into volumetric flasks and made up to volume with copper-free distilled water. Suitable aliquots were taken then for copper

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‡ Elizabeth Arbuthnot Dyson Fellow of the University of Toronto.

§ Metabolic Ward Fellow, Hospital for Sick Children, Toronto.

‖ Playtex Park Research Institute Grantee.

[1] Detailed case histories and clinical courses of these patients will be reported in a forthcoming publication.

[2] Dow-Corning.

determination by the method of Eden and Green (7). Duplicate analyses were performed on all urine and tissue samples and most of the sera. Pairs of blank and standard samples were carried through the digestion procedure with each set of determinations. The confidence limits of the method were calculated according to Copeland (8), on the basis of pairs of parallel determinations on 22 normal serum samples. The confidence limits for a single determination were $3\sigma = \pm 6.21$ μg. per 100 ml. in a range of total copper levels from 89 to 150 μg. per 100 ml. serum.

Ceruloplasmin in the serum was estimated by its paraphenylene-diamine-oxidase (PPD oxidase) activity using the method of Ravin (9). The enzyme activity was expressed as the optical density of the diluted reaction mixture as suggested in the original method (O.D. units).

Radioactive copper studies were carried out in two of the patients (Ron. M. and Mar. Ch.). At the time of these experiments both patients were relatively well, afebrile and stabilized on a low and constant dietary copper (1.3 mg. per day) and moderately high and constant protein and caloric intake. Ron. M. had ascites, which was resistant to various forms of treatment, but was stabilized on a diet containing less than 100 mg. of sodium per day. He had most of his ascitic fluid removed by paracentesis (4,500 ml.) four days before the radioactive copper study. Following this his weight remained stable between 32 and 33 Kg. for the next 10 days.

The studies with radioactive copper were carried out in the manner outlined by Jensen and Kamin (10). After a period of six hours, during which only clear fluids were allowed, an oral dose of copper acetate was given which contained 1 mc. activity in 1.1 mg. (Ron. M.) and 1.3 mg. (Mar. Ch.) copper. Heparinized blood samples were taken thereafter at regular intervals for 60 hours and fractional urine collections obtained for similar time intervals. Stools were pooled for 24 hour periods on three consecutive days with the aid of alternating carmine and charcoal markers.

The packed red cell volume was determined in each blood sample, and this showed no significant variation. Cu^{64} activity was assayed on suitable aliquots of plasma, urine and homogenized 24 hour stool collections in a scintillation well counter. Plasma albumin bound activity was measured in the supernate obtained by high speed centrifugation following precipitation of the globulins by 50 per cent saturation with ammonium sulphate (10, 11). Globulin bound activity was calculated as the difference between total plasma and albumin bound activity.

The method of electrophoresis in a starch supporting medium was also used to assess the relative amounts of Cu^{64} bound to albumin and ceruloplasmin (12). Samples of 5 ml. of serum were used for these experiments and after electrophoretic separation, 1 cm. wide segments of the starch block were placed in perspex tubes and packed tightly by centrifugation. Direct counts were obtained on these preparations. The protein concentration was determined by a modified Folin-Ciocalteau technique (13) and was plotted to compare with the Cu^{64} distribution.

RESULTS

1. Urinary copper excretion

All three patients had cupriuria of a marked degree. The highest and lowest observed 24 hour urinary copper outputs are shown for each patient in Table I. These figures can be compared to those obtained in three control groups: (*1*) children; (*2*) adults, either normal or suffering from various diseases; and (*3*) children suffering from cirrhosis due to causes other than Wilson's disease. It is evident from the figures that the three patients had cupriuria in excess of the controls and in a range reported by others in Wilson's disease (14, 15). Administration of the decoppering agents BAL, ethylenediaminetetraacetic acid (EDTA) or penicillamine produced a marked increase in urinary copper output in all three patients.

TABLE I

*Ranges of urinary copper excretion, serum paraphenylenediamine (PPD) oxidase activity and total serum copper levels in groups of controls and three patients**

	Subjects	Urinary copper excretion		Serum PPD oxidase activity		Total serum copper	
		μg./24 hr.		*O.D. units*		*μg./100 ml.*	
Controls	Children	11–37	(10)	0.385–0.670	(20)	92–150	(12)
	Adults	<40	(10)	0.345–0.555	(11)	88–124	(12)
	Cirrhosis, children	10–100	(14)	0.214–0.612	(8)	63–220	(6)
Patients	Rob. M.	160–375	(8)			123–169	(3)
	Ron. M.	110–590	(25)	0.320–0.470	(6)	116–175	(5)
	Mar. Ch.	160–920	(13)	0.021–0.054	(10)	28–69	(6)

* Figures in parentheses stand for number of individuals tested in the control groups and number of specimens analyzed in the case of the individual patients.

TABLE II

Results of serum ceruloplasmin assays by various methods in groups of controls and patients included in this study

Method	Controls*			Patients		
	Normal	Cirrhosis	Wilson's disease	Rob. M.	Ron. M.	Mar. Ch.
Manometric PPD† oxidase (16),‡ μMole O_2/ml./hr.	3.6 ± 0.73 2.14–5.06	6.7 ± 0.5	0.9 ± 0.5	2.15	2.20	0.39
PPD oxidase standardized against ceruloplasmin (4, 17),§ mg. %	24 16–33		5 0–14		26.6	
Immunochemical (18),‖ mg. %	34 ± 4 27–38	39 ± 10.3 19–56	9 2–19		22.0	4.0

* In the control groups the means and standard deviations are shown and the lowest and highest values obtained in the group, except in the case of normals by the manometric method where the range of ±2 standard deviations is shown.
† PPD, paraphenylenediamine.
‡ Courtesy of Dr. A. G. Bearn, Rockefeller Institute for Medical Research, New York.
§ Courtesy of Dr. I. H. Scheinberg, Albert Einstein College of Medicine, New York.
‖ Courtesy of Dr. G. E. Cartwright, University of Utah, College of Medicine, Salt Lake City.

2. Serum ceruloplasmin levels

It can be seen in Table I that Mar. Ch's. serum had markedly reduced PPD oxidase activity pointing to reduced serum ceruloplasmin level, a finding typical for Wilson's disease (5, 14, 15). Ron. M., the younger of the two brothers, had his serum PPD oxidase activity measured on six occasions within a period of three years. The values fell within the normal range in all except one sample, in which the activity was minimally reduced but still within the range of children suffering from cirrhosis due to causes other than Wilson's disease. The serum PPD oxidase activity of the older brother (Rob. M.) was assayed by a slightly different enzymatic method elsewhere (16).[3] The result fell within two standard deviations from the mean of normals as shown in Table II.

Because of the unusual nature of the above findings their confirmation was sought by other methods (Table II). Both by a manometric enzyme assay (16)[4] and by a sensitive PPD oxidase method calibrated in terms of spectrophotometrically determined ceruloplasmin (17)[5] Ron. M's serum proved to have a ceruloplasmin content within the range of normal. In a third sample of the same patient's serum the amount of ceruloplasmin was measured by a quantitative immunochemical procedure (18).[6] The result, 22 mg. per 100 ml. serum, was only slightly below the normal range of 27 to 38 mg. per 100 ml., obtained by the same method (Table II). In the same sample PPD oxidase activity measured in our laboratory amounted to 0.340 O.D. unit, a level just at the lower limit of our series of controls (Table I).

3. Serum copper levels

Total serum copper levels were also measured and are shown in Table I. Mar. Ch's. serum copper was tested on six occasions; the range of these figures fell below the range of normals, as expected in Wilson's disease (14, 15). The total serum copper levels of the two M. brothers, however, were within the range of normal or higher than normal. Measurements of "direct" and "indirect reacting" copper were not performed but in a single serum specimen of Ron. M. the copper partition could be calculated from the total copper and ceruloplasmin content. The sample contained 116 μg. copper and 22 mg. ceruloplasmin per 100 ml., the latter by immunochemical determination. Based on 0.34 per cent copper in ceruloplasmin, the copper bound to ceruloplasmin was 75 μg. per 100 ml. By subtracting this figure from the total serum copper content of 116 μg. per 100 ml., the derived value of 41 μg. per 100 ml. for nonceruloplasmin bound copper was obtained, which amounted to 35 per cent of the total copper in

[3] Courtesy of Dr. A. G. Bearn, Rockefeller Institute for Medical Research, New York.

[4] Courtesy of Dr. A. G. Bearn, Rockefeller Institute for Medical Research, New York.

[5] Courtesy of Dr. I. H. Scheinberg, Albert Einstein College of Medicine, New York.

[6] Courtesy of Dr. G. E. Cartwright, University of Utah, College of Medicine, Salt Lake City.

the serum. Both these absolute and relative amounts of nonceruloplasmin bound copper in Ron. M's case were higher than Markowitz and associates found in normals (18).

4. Copper content of tissues

The results of the tissue copper analyses are shown in Table III. The copper content of tissues was determined in five children 7 to 13 years of age, who died of causes unrelated to Wilson's or liver disease. The results obtained in these children are very close to those found in adults by Gubler and colleagues (19).

In the case of Rob. M., liver was the only tissue analyzed, the specimen having been obtained as a biopsy at the time of shunting operation. The copper content was 77 μg. per Gm. of wet tissue, a more than seven fold increase over the upper limit of copper content given for normal subjects and more than twice the highest value observed in cirrhosis. In the case of Ron. M. and Mar. Ch. post mortem material was available and several tissues were analyzed. Ron. M's brain contained an excess of copper, compared to brains of both "normals" and patients suffering from cirrhosis. The copper content of his liver and kidney was far in excess of controls; these figures in fact are to our knowledge the highest ever reported in a case of Wilson's disease. The same patient's lung and spleen contained much less copper but it was still in excess of controls. Both the brain and the liver of the third patient (Mar. Ch.) contained an excess of copper, the liver far less than in the other two patients. Her kidney contained normal amounts of copper, as did her lung and spleen. This patient had extensive treatment with BAL and penicillamine in the last two years of her life, which may have influenced these results.

5. Cu^{64} studies

Radioactive copper studies were performed on two patients, Ron. M. and Mar. Ch. The results of the total plasma, the globulin and the albumin bound activity measurements, following the oral administration of Cu^{64}, are plotted against time in Figure 1 and can be compared to values obtained in four normal adults by Jensen and Kamin, in experiments of identical design (10).[7]

The response of the normals to a single oral dose of Cu^{64} had the following characteristics: (*1*) An initial rapid rise in total plasma radioactivity was followed by a steep fall. The peak was reached within the first three hours. (*2*) Starting at six to eight hours following the administration of the dose a secondary rise occurred regularly, which was gradual and was maintained for the whole length of the experiment. (*3*) In three out of the four individuals, 60 hours after the administration of the Cu^{64}, the total plasma activity rose higher than the initial peak. (*4*) The plasma globulin bound activity showed a gradual rise during the whole time of the experiment and this accounted for the secondary rise in total plasma ac-

[7] These data are reproduced with the kind permission of the authors and the Journal of Laboratory and Clinical Medicine.

TABLE III

Copper content of tissues in groups of controls and patients

	Subjects	No. of Cases	Brain	Liver	Kidney	Lung	Spleen
					μg./Gm. wet tissue		
Controls*	Children	5	2.5 1.1–3.3	5.8 3.5–9.6	2.9 1.3–4.9	1.3 1.1–1.5	1.3 0.6–2.3
	Adults†	5	6.3 5.1–8.3	5.3 3.0–9.5	2.2 1.8–3.1	1.2	1.0 0.7–1.1
	Cirrhosis† (adults)	12	6.5 3.5–10.2	9.4 3.4–29.7	2.2 1.6–3.5		1.1 0.9–1.5
Patients	Rob. M.			77			
	Ron. M.		24	482	226	11	7.4
	Mar. Ch.		57	29	2.7	1.4	0.9

* In the control groups the means and the range are shown.
† Gubler and associates (19).

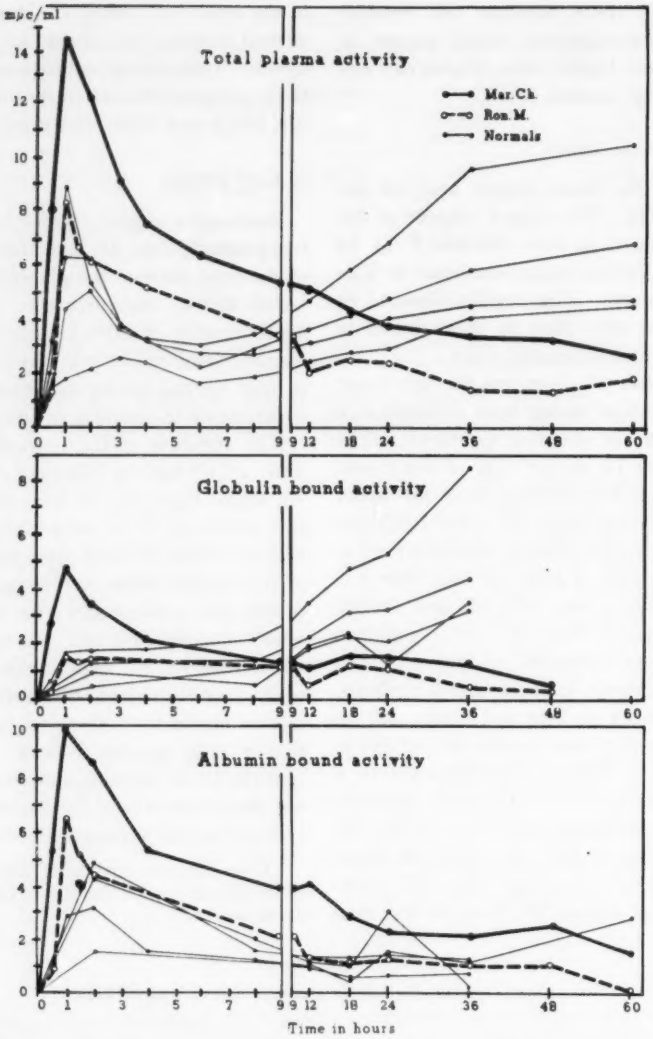

FIG. 1. TOTAL PLASMA, ALBUMIN AND GLOBULIN BOUND ACTIVITY PLOTTED AGAINST TIME FOLLOWING ADMINISTRATION OF SINGLE ORAL DOSE OF Cu^{64}

tivity. (5) The albumin bound activity showed an early peak which was followed by gradual decline of the activity in this fraction. The albumin bound activity accounted for the initial peak of the total plasma activity (10, 11, 20).

The patient Mar. Ch., exhibiting all the clinical and biochemical characteristics of Wilson's disease including a markedly reduced serum ceruloplasmin level, responded to the oral administration of a single dose of Cu^{64} in a manner typical for Wilson's disease (10, 11, 20). In her case the initia. peak of total plasma activity was not followed by a secondary rise, but a gradual fall. The albumin bound activity closely paralleled the curve of total plasma activity and the globulin bound activity showed an initial rise followed by a gradual fall instead of the gradual and sustained rise typical for control subjects (Figure 1). This type of re-

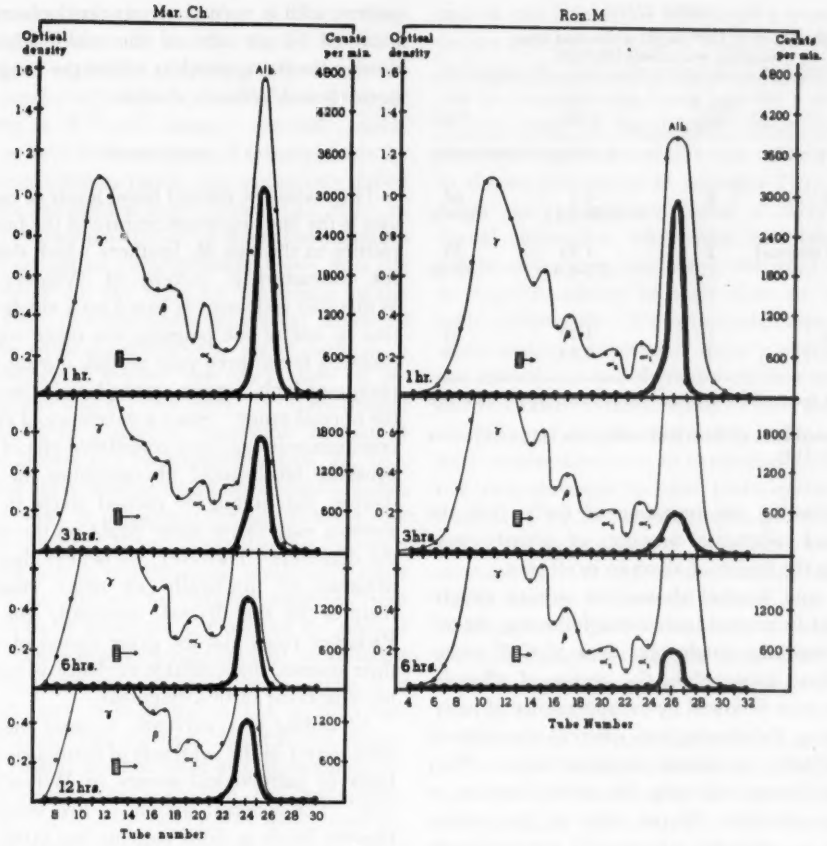

FIG. 2. ELECTROPHORETIC DISTRIBUTION OF Cu^{64} ACTIVITY FOLLOWING ADMINISTRATION OF SINGLE ORAL DOSE OF Cu^{64}

The heavy lines connecting the closed circles show the distribution of radioactivity. The protein distribution curve is represented by the light lines connecting open circles. A cross-hatched box marks the area of the origin and the arrow points in the direction of migration. The time at which the sample was drawn following the administration of Cu^{64} is shown at the lower left corner of the individual plots.

sponse in cases of Wilson's disease does not suggest incorporation of Cu^{64} into ceruloplasmin but was shown by Bush and co-workers to be due to contamination of the 50 per cent ammonium sulfate precipitate with albumin bound Cu^{64} (11).

The results obtained in the case of Ron. M. were of special interest because this patient had a normal serum ceruloplasmin level. In spite of this, he gave a response similar to Mar. Ch. and other cases of Wilson's disease. There was no secondary rise in his total plasma activity and no rise in globulin bound activity but a gradual slow fall following a slight initial rise (Figure 1).

The electrophoretic distribution of the protein bound Cu^{64} activity, observed in the course of the above two experiments, is shown in Figure 2. In the case of Mar. Ch., no measurable amount of radioactivity appeared in the α_2 globulin fraction which contains ceruloplasmin, in sera collected 1, 3, 6 and 12 hours after oral administration of Cu^{64}. Similarly in Ron. M. activity did not appear in the α_2 globulin of sera collected 1, 3, and 6

TABLE IV

Excretion of Cu^{64} in the urine and stool following oral administration

	No. of cases	Urine	Stool
		% of orally administered dose	
Controls*			
Normal†	8	0.1 0.05–0.12	67 45–94
Wilson's disease†	7	1.93 0.2–4.4	55 36–85
Patients			
Mar. Ch.	1	1.12	34
Ron. M.	1	0.83	60

* In the control groups the means and the range are shown.

† Combined data of Bush and colleagues (11) and Jensen and Kamin (10).

hours following the ingestion of Cu^{64}. On the other hand substantial amounts of activity were present in the region of albumin in all sera.

Bearn and Kunkel showed in similar experiments that in normal individuals following the administration of a single oral dose of Cu^{64} radioactivity first appeared in the region of albumin. This was soon followed by an appearance of activity in the α_2 globulin region, whereas the albumin bound activity decreased simultaneously. They found six hours following the administration of Cu^{64} approximately 50 per cent of the activity bound to α_2 globulin, presumably ceruloplasmin (21). This shift of copper from albumin into ceruloplasmin did not occur in cases of Wilson's disease studied by Bearn and Kunkel (21) nor did it occur in our two cases of Wilson's disease (Mar. Ch. and Ron. M.).

Urinary and fecal excretion of Cu^{64} was also measured in both Ron. M. and Mar. Ch. for three days following the oral administration of Cu^{64}. It was found previously by others (10, 11, 20) that patients suffering from Wilson's disease excrete a higher percentage of a single oral dose of radioactive copper in the urine and a lower percentage in the stool than normals. As can be seen in Table IV both Mar. Ch. and Ron. M. excreted a much higher percentage of the administered Cu^{64} in the urine than did normals. Their values fit into the range found in Wilson's disease (10, 11, 20). Mar. Ch. excreted less Cu^{64} in the stool than did normals in similar experiments. Ron. M., the patient with a normal serum ceruloplasmin level, excreted 60 per cent of the orally administered dose in the feces, which is within the range of both normals and Wilson's disease.

DISCUSSION

The finding of normal blood levels of ceruloplasmin is the one important feature of the biochemical picture in the two M. brothers which does not fit the characteristic pattern of Wilson's disease. While this statement is based on a single observation in one of the patients, the other was closely followed for a three year period, during which he maintained his serum ceruloplasmin level within the normal range. Since a deficiency of circulating ceruloplasmin has been considered one of the most constant biochemical abnormalities in Wilson's disease, the finding of normal levels in our two patients may throw some doubt on the validity of the diagnosis. However, we believe that with the presence of practically all other characteristic features of the disease, especially the Kayser-Fleischer rings and the great excess of copper in their tissues, the available evidence is overwhelmingly in favor of this diagnosis.

According to a currently favored theory a genetic defect in the synthesis of ceruloplasmin is the basis of pathological events in Wilson's disease (4). The constant finding of low serum ceruloplasmin levels in these patients has fitted well into this theory. Probably the most convincing evidence was furnished by Scheinberg and associates who found two young patients in whom a profound deficiency of ceruloplasmin antedated all other biochemical, pathological and clinical manifestations of Wilson's disease (22, 23, 24). One of these patients was only 10 months old when he was discovered to have no measurable amounts of ceruloplasmin in the blood. The diagnosis in this patient has not at this time been confirmed beyond doubt, but it was quite probable in view of the finding of an excessive amount of copper in his liver at four years of age and the definite diagnosis of Wilson's disease in one of his sibs (23, 24). The virtual absence of ceruloplasmin from this patient's blood at 10 months of age suggests that a deficiency of ceruloplasmin is not only a constant feature of Wilson's disease but may be present as early as during the first year of life.

There are, however, a number of observations which are difficult to fit into this theory. First, it has not been explained adequately how the deficiency in ceruloplasmin leads to an increased copper absorption in Wilson's disease. Secondly, maintenance of normal blood levels of ceruloplasmin by intravenous administration has apparently failed to produce biochemical improvement (25, 26). In addition, the administration of estrogens resulting in a rise of serum ceruloplasmin levels into the normal range did not influence the course of the disease (1). Furthermore, in several unrelated conditions such as nephrosis, sprue and in a rather ill-defined copper deficiency state in infants, ceruloplasmin deficiency of considerable duration and of a degree comparable to that of Wilson's disease has been observed (18, 27, 28). In none of these disease states does ceruloplasmin deficiency lead to manifestations of Wilson's disease. In addition, it was found that in Wilson's disease the serum ceruloplasmin levels vary quite widely from patient to patient, ranging from "none" to more than fifty per cent of the normal (4, 18). Thus it was recognized that in Wilson's disease the deficiency of ceruloplasmin was of a lesser and more variable degree than in other disease states based on a genetic defect in the formation of a plasma protein, such as congenital agammaglobulinemia, afibrinogenemia and hemophilia. Moreover, correlation could not be established between the degree of ceruloplasmin deficiency and the severity of the disease (18).

According to the findings presented in this paper, a patient suffering from Wilson's disease may even have normal amounts of ceruloplasmin in the serum. To our knowledge this has not been reported in an authentic case of Wilson's disease although Dr. Bearn has kindly communicated to us that he has made the same observation in two other proven patients (29).

The presence of normal amounts of ceruloplasmin in our two patients cannot be related to the fact that they have failed to show signs of neurological involvement. We have observed extremely low levels of ceruloplasmin in two other cases of hepatic Wilson's disease and others have made similar observations (18, 29).

In trying to explain the finding of normal blood levels of ceruloplasmin in a case of Wilson's disease the argument can be raised that environmental factors may have either stimulated a genetically defective synthetic process or slowed the rate of degradation of ceruloplasmin, leading thereby to a rise in ceruloplasmin levels into the normal range at some stage of the disease. Bearn has shown that in Wilson's disease this may occur in response to the administration of estrogen (1). Our patients had gynecomastia and a delay in male sexual maturation, which may have been the result of increase in circulating estrogens secondary to impaired ability of their liver to metabolize such compounds. Unfortunately, measurements of circulating estrogens or urinary excretion were not carried out and therefore no firm conclusions can be drawn in this respect. However, in cases of Wilson's disease with comparable degree of liver involvement, rise in ceruloplasmin levels has not been observed by other investigators. Moreover, Ron. M. had normal levels of ceruloplasmin before the appearance of the signs of estrogenic overactivity. Whatever the reasons may be for the maintenance of normal ceruloplasmin levels in these patients, there remains the question of why the disturbances of copper metabolism persisted and the disease progressed in the absence of a deficiency in ceruloplasmin.

The finding of marked delay in the incorporation of Cu^{64} into ceruloplasmin in Ron. M. may suggest that an abnormality in the metabolism of ceruloplasmin still existed in this patient, which, however, did not affect the blood levels of ceruloplasmin. In connection with this it is of interest to note that Sternlieb, Morell and Scheinberg found no increase in the rate of incorporation of Cu^{64} into ceruloplasmin in a patient with Wilson's disease in whom blood levels of ceruloplasmin were raised into the normal range by intravenous infusion (26).

It was shown previously by others that in Wilson's disease, compared to normals and other types of cirrhosis, there is a marked delay in the incorporation of copper into ceruloplasmin (10, 11, 20). Since there is no evidence to show that copper bound to ceruloplasmin exchanges with ionic copper *in vivo*, these findings could be interpreted as evidence of a defective synthesis of ceruloplasmin. This interpretation fitted in very well with the usual marked reduction in circulating ceruloplasmin in Wilson's disease. In view of these considerations the present findings in Ron. M's. case

are extremely difficult to interpret. In this patient a marked delay in the incorporation of copper into ceruloplasmin was observed in the presence of normal blood levels of ceruloplasmin. If we accept that the delay in the incorporation of Cu^{64} is evidence of a decreased rate of synthesis, the normal concentration of ceruloplasmin in the blood could only have been maintained by a simultaneous decrease of a similar degree in the rate of decay of ceruloplasmin. This seems highly unlikely and to our knowledge such a mechanism has not been proven to exist in the case of any other plasma protein.

Scheinberg and Morell (17, 30) have shown recently that contrary to previous belief, the copper-protein bond in ceruloplasmin can be reversibly dissociated under certain rather unphysiological conditions *in vitro*. Should this occur *in vivo*, indicating that ceruloplasmin may reversibly release and bind copper at various sites in the body, the rate of incorporation of Cu^{64} into ceruloplasmin may reflect the rate of this exchange process as well as synthesis of ceruloplasmin. Consequently the finding of a delay in the incorporation of Cu^{64} into ceruloplasmin in the presence of normal blood levels of ceruloplasmin could point to a disturbance of this hypothetical exchange process. This explanation, however, cannot as yet be seriously considered, since there is no proof whatsoever of the physiological occurrence of copper exchange in ceruloplasmin.

Finally, we have to consider a suggestion first made by Bush and colleagues (11). According to this the failure to measure incorporation of Cu^{64} into ceruloplasmin in Wilson's disease could be due to the fact that the administered Cu^{64} is markedly diluted into the tremendously expanded copper pool of these patients. This suggestion was made following an observation very similar to ours. They found that in a case of Wilson's disease with a moderately reduced serum ceruloplasmin level of 19 mg. per 100 ml. (normal 27 to 38 mg. per 100 ml.) the uptake of labeled copper into ceruloplasmin was just as much delayed as in patients with barely measurable amounts of ceruloplasmin in the serum. Our patient (Ron. M.) certainly had an expanded copper pool as evidenced by an excess of nonceruloplasmin bound copper in his serum and the large copper deposits found in his tissues at the time of death, four months after the radioactive copper studies were performed. Therefore it is possible that our failure to measure incorporation of copper into ceruloplasmin was primarily due to considerable dilution of the isotope.

It will be evident at this point that the observations presented in this study are difficult to interpret. The finding of normal blood levels of ceruloplasmin in two otherwise typical cases of Wilson's disease suggests that a defect in the production of ceruloplasmin may not be the basic metabolic abnormality in this condition, or at the least not in all cases. Nevertheless, the observation of a marked delay in the rate of incorporation of copper into ceruloplasmin in one of these patients may still suggest the presence of an abnormality in the metabolism or function of ceruloplasmin. Concerning this latter point, however, no firm conclusions can be drawn until the real reasons for the generally observed delay in copper uptake into ceruloplasmin in Wilson's disease are clarified by further experimental work.

The observations presented here do not have direct bearing on other theories concerning the pathogenesis of Wilson's disease, especially the theory advanced by Iber, Chalmers and Uzman (31), and therefore no comments can be made in this respect.

In spite of the wealth of new knowledge and a multitude of metabolic studies, the nature of the basic defect in Wilson's disease remains uncertain. One is increasingly impressed by the wide variation of both the clinical and metabolic findings in this condition. Are these all secondary changes and is the primary expression of the basic defect still undiscovered? Or do these variations represent a series of similar syndromes produced by a series of alleles varying among themselves? These are open questions requiring further investigation and especially more detailed family studies, including families with consanguineous parentage.

SUMMARY

1) In two brothers suffering from Wilson's disease normal blood levels of ceruloplasmin were found.

2) Detailed investigation of the two patients revealed the presence of practically all other typical features of Wilson's disease.

3) One of the patients, in spite of the presence of normal blood levels of ceruloplasmin, showed a marked delay in the rate of incorporation of Cu^{64} into ceruloplasmin.

4) The significance of these findings was discussed and related to a currently held theory concerning the pathogenesis of Wilson's disease.

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FAMILIAL INCREASE IN THE THYROXINE-BINDING SITES IN SERUM ALPHA GLOBULIN *

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Thyroxine in human serum migrates during paper electrophoresis at pH 8.6 with at least three protein components: an α globulin intermediate between α_1 and α_2, thyroxine-binding protein (TBP) (1, 2), albumin (1, 2) and a component moving faster than albumin, prealbumin or thyroxine-binding prealbumin (TBPA) (3). Alterations in serum protein-bound iodine (PBI) may occur in certain circumstances without concomitant hyper- or hypothyroidism, but with parallel changes in the thyroxine-binding capacity of TBP. Examples are the elevated PBI and TBP capacity in pregnancy (4, 5) and after estrogen therapy (6), and the depressed PBI and TBP capacity produced by methyl testosterone (7). It seemed logical, therefore, to look for alterations in TBP in patients with abnormal serum PBI levels in whom other evidence of thyroid dysfunction was lacking. We wish to report the occurrence of an elevated PBI and TBP capacity in an otherwise normal adult male and in one of his three children.

CASE REPORT AND METHODS

W.W. was a male 48 years of age when seen on March 17, 1958, because his serum PBI was found to be "elevated" on a routine periodic health examination. He was asymptomatic and gave no history of thyroid disease in himself or in his family. He had never received radiographic contrast media and there was no known ingestion of other organic iodine compounds. He gave no history of diseases known to alter the serum proteins. His libido and potentia were considered to be normal. His physical examination was completely normal. No goiter or evidence of hyper- or hypothyroidism was evident. Hair growth and distribution and genitalia were normal.

His two sons, aged 18 and 12 years, and his daughter, aged 15 years, also presented no abnormal findings by medical history or physical examination except for the history of allergic asthma treated with potassium iodide in the 12 year old boy. The girl gave a normal menstrual history with onset at 12.5 years of age.

Pertinent laboratory findings on the father are presented in Table I. Normal values and references to the published techniques used in obtaining these laboratory data are also presented in this table.

Measurement of the thyroxine-binding capacity of TBP was done as before (8, 9) except that electrophoresis was carried out in 0.1 M ammonium carbonate as well as in barbital buffer. The pH of the solution of U.S.P. grade ammonium carbonate was adjusted to about pH 8.4 by bubbling CO_2 through it for a few minutes. The pH on the paper strip at the end of the run was approximately 8.5 as determined by indicator paper. The thyroxine-binding capacity of TBP determined in ammonium carbonate is the same as when barbital is used (9). Electrophoresis in the former, however, results in a large additional thyroxine peak running ahead of albumin similar to that found with "Tris" maleate buffer (3). In this study, two levels of added I^{131}-labeled L-thyroxine were employed: 2.69 μg. per ml. and 1.34 μg. per ml. The former was done in triplicate and the latter in duplicate.

RESULTS

The findings in the father are given in Table I. The serum PBI and serum butanol extractable iodine (BEI) were elevated to two to three times the mean value found in normal individuals. The I^{131}-labeled triiodothyronine erythrocyte uptake (I^{131} T^3 RBC uptake) which is reported to increase in hyperthyroidism (12, 22) was less than half of the lower limit of published normal values. The basal metabolic rate (BMR) and basal pulse rate were at the low normal to hypothyroid range. The serum cholesterol and I^{131} thyroid uptake were well within the normal range.

The disappearance of intravenously injected I^{131} L-thyroxine from the plasma (expressed in Table I as half time, $T_{1/2}$, and as fraction of the thyroxine pool turned over per day) was slower than the normal mean by almost a factor of two and slower than reported in myxedema (14). The extrathyroidal organic iodine pool (EOI) was

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TABLE I

Familial increase in thyroxine-binding sites in serum alpha globulin: Pertinent laboratory findings in the father

Laboratory tests			Time in months (1958)					
Name	Reference to method	Normal values	Mar.	Apr.	May	June	Nov.	Dec.
PBI (μg. %)	(10)	4.0–7.4	13.9	16.0			11.8	14.7
Inorganic iodine (μg. %)		0.5–3.0	0.9					
BEI (μg. %)	(11)	3.4–6.8		13.0				12.3
I^{131} T^3 RBC (% uptake @ 2 hrs.)	(12)	10.0–17.0			4.8			
I^{131} thyroxine plasma T_4 (days)	(13, 14)	6.6 ± 0.7						12.2
Thyroxine turnover rate (%/day)	(13, 14)	10.5 ± 1.1						5.7
Extrathyroidal organic iodine pool (μg.)	(13, 14)	548 ± 107						1,099.0
Thyroxine degradation rate (μg./day)	(13, 14)	57 ± 11						63.0
BMR (%)		−15–10	−20					
Basal pulse rate		60–90	60					
Serum cholesterol (mg./100 ml.)	(15)	125–325	212					
I^{131} thyroid uptake (% of dose)	(16)	10.0–36.0 (24 hrs.)	1 hr. = 4.2 2 hr. = 6.6 6 hr. = 10.0 24 hr. = 21.6					
Total serum proteins (Gm. %)	(17)	6.5–8.5				7.6		
Serum albumin (Gm. %)		4.0–5.5				4.8		
Serum globulin (Gm. %)		1.5–3.5				2.8		
BSP (% retention at 45 min.)	(18)	0–6.0				2.3		
17-Ketosteroids (mg./24 hrs.)	(19)	6.0–20.0			19.5			
Urinary estrogens (rat units/24 hrs.)	(20)	0–4.0			10.0	5.0		
Follicle stimulating hormone (mouse units/24 hrs.)	(21)	6.0–48.0			6.0	Neg. at 3 M.U.		

about twice as large as the normal mean and comparable to that in thyrotoxicosis (14). The degradation rate of thyroxine in micrograms per day was, however, within the normal range.

Normal values were found for serum proteins, bromsulfalein retention, and urinary 17-ketosteroid excretion. The slightly elevated values for 24 hour urinary estrogen excretion were of questionable significance, as was the low value for urinary follicle stimulating hormone.

TABLE II

Familial increase in the thyroxine-binding sites in serum alpha globulin: Laboratory findings in children of propositus

Child	Age	Sex	PBI	Inorganic iodine	BEI	I^{131} T^3 RBC uptake	BMR
	yrs.		*μg. %*	*μg. %*	*μg. %*	*per cent*	
1	18	M	5.8	<10	5.4	10.7	−6
2	12	M	>25	500	6.2	8.4	
3	15	F	10.1	<10	9.1	4.5	−6

TABLE III

*Thyroxine-binding capacity**

Subject	Thyroxine-binding capacity of TBP		Thyroxine in prealbumin
	Added T_4 = 1.34 μg./ml.	Overall average†	Added T_4 = 2.69 μg./ml.
	μg. T_4/ml.	μg. T_4/ml.	μg. T_4/ml.
Normal A	.22	.24	1.2
Normal B	.16	.21	1.2
Father (Nov.)	.47	.45	1.3
Child 1	.14	.14	1.5
Child 2	.23	.20	1.1
Child 3	.36	.29	1.5

* Determinations made by electrophoresis in 0.1 M ammonium carbonate.

† Overall average of five determinations, two at 2.69 μg. per ml. T_4 added and three at 1.34 μg. per ml. T_4 added.

Table II summarizes a few pertinent laboratory findings on the children of the propositus.

The serum PBI and BEI were definitely elevated in the daughter although not so high as in the father. The low I^{131} T^3 RBC uptake in the daughter was approximately the same as in the father. The daughter's normal BMR value agreed with the clinical impression that she also was euthyroid. The serum of the second son was contaminated by relatively large quantities of inorganic iodine which apparently resulted in elevation of the serum PBI. The spurious nature of this elevation was borne out by the normal BEI. The slight depression of the I^{131} T^3 RBC uptake value in the second son must be ascribed to contamination with iodide (*22*) until proved otherwise.

Values obtained with ammonium carbonate for the thyroxine-binding capacity of TBP in the members of the family, as well as in two normal adults who were studied simultaneously, are listed in Table III. In addition to the overall averages for five determinations, the averages for duplicate

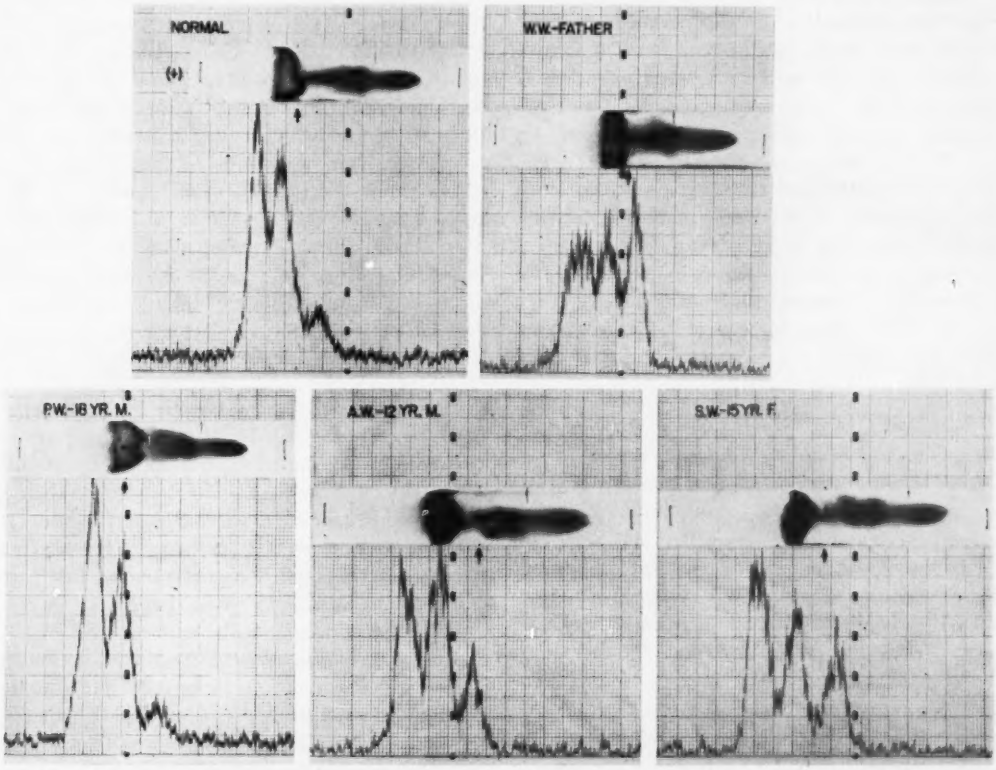

FIG. 1. ELECTROPHORESIS OF I^{131} THYROXINE-SERUM MIXTURES IN 0.1 M AMMONIUM CARBONATE

Each serum mixture contained 1.34 μg. of added L-thyroxine per ml. The paper strips, stained with bromphenol blue, are mounted above their radioactivity records. The arrows indicate the points of application. Electrophoresis was by the reverse-flow technique.

determinations at an added thyroxine concentration of 1.34 μg. per ml. are given. The latter are considered to be more accurate since the area under the TBP peak at the higher thyroxine level (2.69 μg. per ml.) was very small and its measurement was subject to greater error. The two averages agree closely except in Normal B and Child 3.

The values for the normals were in the range previously reported for normal adults (8). The results in Child 1 and Child 2 were also normal, but TBP in sera from the father and Child 3 had greater than normal capacity to bind thyroxine. The highest value was found in the father. Thyroxine-binding capacities determined with barbital buffer gave similar results. Representative electrophoretic patterns are presented in Figure 1 and demonstrated no abnormality other than the quantitative difference already noted.

Table III also gives values for the quantity of thyroxine associated with TBPA at the higher thyroxine level. In the case of Normal A, thyroxine in TBPA was determined at a series of thyroxine concentrations. The results indicated that, although a plateau was approached at the 2.69 μg. per ml. level, it had not been reached. The results in Table III are probably lower, therefore, than the capacity of TBPA for thyroxine, but they permit comparison between the normals and the subjects of this study. The significance of the rather small differences observed is uncertain, but it is noteworthy that the value in the father did not differ from that in the normals.

DISCUSSION

This report describes the occurrence of an elevation of the thyroxine-binding capacity of TBP in an appraently euthyroid man with an otherwise unexplained elevation of serum PBI and BEI. No cause for this alteration was apparent. The finding of a similar abnormality in one of his children, however, suggested that it might have a genetic basis. The evidence presented does not distinguish between an increase in the concentration of TBP itself or in available sites on the TBP molecule as a cause for the increased binding capacity. There appeared to be no alteration in the binding of thyroxine by TBPA in the affected subjects or of any qualitative alteration in the electrophoretic distribution of thyroxine. Chemical identification of the serum PBI was not done, but in the presence of an elevated thyroxine-binding capacity it is reasonable to assume that it was thyroxine.

If, as seems likely, the increase in TBP capacity was the primary alteration, it is of interest to speculate on how the other observed abnormalities in thyroid physiology might have come about. If it is assumed that thyroxine (or triiodothyronine) combined with serum protein is unavailable to the tissues and that the metabolic action of the hormones is exerted only by the diffusible free thyroxine, which is in equilibrium with the bound, then a higher total thyroxine level in serum would be required for maintenance of euthyroidism (2). A normal thyroid-pituitary system would result, therefore, in an elevated serum PBI together with an expanded extrathyroidal thyroxine pool, as observed in the case presented. In the euthyroid steady state, however, the quantity of thyroxine degraded per day would be normal, so that the fraction of the total thyroxine pool turning over per day would be decreased. In this steady state the function of the thyroid gland would be no different from normal. The findings presented are compatible with such an hypothesis.

The depression in I^{131} T^3 RBC uptake may also be explained by the increase in TBP capacity for thyroxine since triiodothyronine and thyroxine interact with the same sites on this protein (23). From the expression for an equilibrium reaction,

$$k\ (\text{free thyroxine}) = \frac{(\text{thyroxine bound to TBP})}{(\text{unoccupied TBP sites})}.$$

If free thyroxine remains constant in the presence of an increase in total TBP sites and total serum thyroxine, then the concentration of unoccupied TBP sites must increase. The addition of a small increment in labeled thyroxine (or triiodothyronine) to such an altered serum would result in a greater normal proportion of the labeled substance being bound to TBP. There would then be less free *labeled* hormone available for uptake by the red blood cells even though the total quantity of hormone taken up might be normal. In the absence of other alterations affecting RBC uptake, the changes in TBP and PBI in the patient would, therefore, explain the lowered I^{131} T^3 RBC uptake.

The findings suggest that some of the instances of elevated serum PBI levels without accompany-

ing hyperthyroidism, which are found sporadically and are usually attributed to contamination with nonhormonal iodine (24), may be the result of an increase in the thyroxine-binding capacity of serum TBP. Tanaka and Starr (25) described 10 such patients, all of whom were women. The present report is, to our knowledge, the first indication that such an abnormality may be familial. The frequency of this abnormality is unknown. It is not unlikely that the more frequent application of the test of I^{131} T^3 RBC uptake, even though subject to alteration by factors unrelated to TBP, may serve to bring such cases to attention. Measurement of TBP capacity in suspected cases would, of course, be a more direct approach.

It is also possible that alterations in TBP capacity can explain other paradoxical deviations in the PBI level. Tanaka and Starr (25) apparently detected instances of euthyroidism with low PBI and low TBP capacity, although they grouped these patients with subjects usually considered to be in the normal range of PBI. It might be reasoned, also, that an elevated or depressed TBP capacity could explain the occurrence of a normal PBI in clinical hypothyroidism or hyperthyroidism, respectively. We know of no examples of this.

It should be emphasized that TBP is not the sole controlling factor in extrathyroidal distribution and metabolism of thyroid hormone. Alterations analagous to those described could result from changes in thyroxine binding by serum albumin or prealbumin and by factors influencing the peripheral degradative or metabolic processes. Further study is required to distinguish between these possibilities.

SUMMARY

Because the serum protein-bound iodine (PBI) has been found elevated in euthyroid states associated with an increase of thyroxine-binding capacity of plasma thyroxine-binding α globulin (TBP) due to known causes, a search was made for individuals with unexplained elevation of the serum PBI. A euthyroid man was found with serum PBI and butanol extractable iodine (BEI) levels ranging between two and three times normal and maintained over an observation period of nine months. A subnormal I^{131} triiodothyronine erythrocyte uptake (I^{131} T^3 RBC uptake) suggested that the serum PBI elevation was related to increased affinity of the plasma proteins for thyroid hormone. This suspicion was confirmed by the finding of an increase in thyroxine-binding capacity of TBP by reverse-flow serum electrophoresis. No abnormality was detected in thyroxine binding to albumin or prealbumin. Although the extrathyroidal organic iodine pool was expanded to roughly the same size as that found in patients with untreated thyrotoxicosis, the degradation rate in micrograms of thyroxine per day was within the normal range. The finding that one of this man's three children also had elevated serum PBI and BEI levels, a subnormal I^{131} T^3 RBC uptake and an elevated TBP capacity constitutes evidence that the abnormality is familial and could conceivably be hereditary. No other cause for this abnormality was detected. The finding of such markedly increased levels of thyroid hormone in the serum of euthyroid individuals supports the contention that the total serum thyroxine concentration does not alone govern the metabolic status of the individual.

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EFFECTIVENESS OF TRANSFUSIONS OF FRESH AND LYOPHILIZED PLATELETS IN CONTROLLING BLEEDING DUE TO THROMBOCYTOPENIA *

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Transfusion of platelets into thrombocytopenic recipients results in the control of hemorrhage if the infused platelets are viable and circulate in the recipient's blood in sufficient numbers (1, 2). Storage by commonly employed methods for brief periods alters platelets so that they will not circulate when infused (3). Infusions of platelets preserved by freezing (4) and lyophilization (5) have not resulted in increase of recipient's platelet levels although such infusions have been reported to control bleeding due to thrombocytopenia. The effectiveness was assayed by observing the degree of clinical bleeding in thrombocytopenic recipients before and after the infusion of preserved platelets (4, 5). However, such observations are difficult to quantify because bleeding in thrombocytopenic individuals is variable and intermittent. Some individuals with severe thrombocytopenia have no abnormal bleeding. Others with the same degree of thrombocytopenia may have serious and even fatal hemorrhage.

Thrombocytopenia is the major cause of bleeding in animals exposed to whole body irradiation (6). A characteristic of the hemorrhagic state following such irradiation is the appearance of large numbers of red blood cells in the lymph (7). Transfusions of viable platelets that circulated in recipients suppressed the number of red blood cells in thoracic duct lymph of irradiated dogs (8). In the present studies, the effectiveness of lyophilized platelets was compared with the effectiveness of fresh platelets in suppressing the number of red blood cells in the thoracic duct lymph of irradiated dogs.

MATERIALS AND METHODS

Experimental animals. Adult mongrel dogs were housed in individual metabolism cages and fed Purina Dog Chow with a daily supplement of meat. Four dogs (430, 431, 440 and 441) received chlortetracycline daily *per os* beginning seven days after irradiation. Three dogs (426, 428 and 435) were given penicillin and dihydro-streptomycin, and one (442) was given chlortetracycline on the day of operation (cannulation of thoracic duct).

Technique of irradiation. Five dogs (426, 428, 430, 431 and 441) received 500 r. whole body irradiation, and three (435, 440 and 442) received 550 r. All of the dogs were anesthetized with pentobarbital sodium during irradiation. Radiation was delivered by a General Electric Maxitron X-ray machine operating at 250 KVP, 30 ma. and filtered with 0.5 mm. of copper and 1.0 mm. of aluminum. The target-skin distance was 110 cm. The dose rate at the skin surface was 27 r. per minute. Half of the total dose was delivered to each side of the body.

Cannulation of thoracic duct. The thoracic duct was cannulated between the tenth and seventeenth day following irradiation after the dogs became thrombocytopenic. One hour before surgery the animals were given approximately 100 ml. of corn oil *per os* to facilitate identification of the lymph channels. Pentobarbital sodium was administered intravenously for anesthesia, and 5 per cent glucose in 0.85 per cent sodium chloride solution was infused during the operation. The operative procedure of Ross, Furth and Bigelow (7) was employed, in which plastic (polyethylene) cannulae are introduced into the thoracic duct and into a branch of the jugular vein. The free ends of both of these cannulae were exteriorized. When the lymph was not being sampled, the free ends of the two cannulae were connected and the lymph allowed to flow into the venous system.

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Collection of and observations on lymph. Lymph was collected from the free end of the cannula in the thoracic duct, and the total output of lymph (ml. per minute) was measured. When the periods of sampling were prolonged, the dogs were maintained under pentobarbital sodium anesthesia and were given an intravenous infusion of 5 per cent glucose in 0.85 per cent sodium chloride solution. The rate of infusion was adjusted to approximate the rate of output of lymph.

Red blood cells in lymph samples were counted with an electronic counter.[1] All samples were diluted with 0.85 per cent sodium chloride solution so that the cell concentration in the 0.5 ml. metered fell between 10,000 and 90,000. The reproducibility of results with this counter is within approximately ± 2 per cent of the mean (9, 10).

Results are expressed as the total output of red blood cells in the lymph per unit of time. These values were calculated as follows:

$$\frac{\text{ml. of lymph}}{\text{min. of collection}} \times \frac{\text{number of RBC}}{\text{ml. of lymph}} = \frac{\text{number of RBC}}{\text{min. of collection}}.$$

Hematologic studies. Hematocrit values were determined by a microtechnique (11). Leukocytes were counted in blood diluted with 2 per cent acetic acid. A dilution of 1:20 was used until the total count fell below 1,000 per cu. mm., and then a dilution of 1:10 was used. Platelets were counted by the phase contrast technique of Brecher and Cronkite (12). Studies of blood coagulation were performed using previously described techniques (13).

Preparation of fresh platelet suspensions. Healthy donor dogs were anesthetized with thiopental sodium and blood was obtained from femoral artery punctures. The method (14) for the separation and concentration of the platelets was based on differential centrifugation and included the use of nonwettable equipment with disodium ethylenediaminetetraacetatic acid (EDTA) as the anticoagulant. The final suspension of platelets was usually completed within four to five hours of drawing the blood. The platelet suspensions were infused through plastic recipient sets with nylon filters.

Preparation of lyophilized platelets. Donor dogs were bled as for the preparation of fresh platelet suspensions. The technique of Klein and associates (5) was used for separation, concentration and lyophilization of the platelets. The only modifications were: (*a*) silicone coated glassware was used in place of plastic blood bank equipment, (*b*) Triton® was not used, and (*c*) the final suspension of platelets before lyophilization was in 0.85 per cent sodium chloride solution containing 1 per cent EDTA.

The final platelet concentration before lyophilization was adjusted to 10,000,000 platelets per cu. mm. The lyophilized platelets were stored at −20° C. for 5 to 21 days. The lyophilized material was reconstituted 30 minutes before use by addition of sterile, distilled water in an amount equal to the volume of the final platelet suspension before lyophilization. The reconstituted material was infused through plastic recipient sets with nylon filters. On the assumption that the recipient's blood volume was 7 per cent of body weight, infusion of 1.40 ml. of this material per Kg. body weight would be expected to raise the recipient's platelet level by approximately 200,000 per cu. mm. if the infused platelets circulated.

[1] Coulter Counter, Model A; Coulter Electronics, 5227 N. Kenmore, Chicago, Ill.

RESULTS

Hematologic studies

The changes in the leukocyte, hematocrit and platelet levels in the eight dogs were similar to those described previously by many investigators after amounts of whole body irradiation comparable to the amounts employed in these experiments. The mean control leukocyte count was 16,700 per cu. mm. By the sixth to eighth postirradiation day the leukocyte level was < 1,000 per cu. mm. in each of the irradiated animals. The mean control platelet count was 339,000 per cu. mm. and the mean control hematocrit value was 44.6 per cent. By the eleventh postirradiation day, the platelet level was < 10,000 per cu. mm. in each of the dogs and the mean hematocrit value was 32.5 per cent.

Effects of infusion of lyophilized platelets alone

Three dogs (430, 440 and 441) were given one or more infusions of lyophilized platelets but no fresh platelets.

Dog 430 was given 500 r. whole body irradiation. Cannulation of the thoracic duct was carried out 14 days later. The peripheral blood platelet count on the day of operation was < 5,000 per cu. mm. The initial lymph samples were bloody and observations on the lymph were begun upon com-

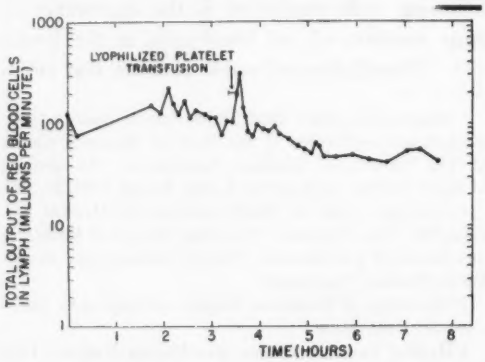

FIG. 1. 500 r. WHOLE BODY IRRADIATION (DOG 430)

pletion of the operation. Results are shown in Figure 1. The output of red blood cells in the lymph appeared stable over a three and a half hour control period. Eighteen ml. (1.73 ml. per Kg.) of lyophilized platelets then was infused over a nine minute interval. No increase in the recipient's circulating platelet level occurred following this infusion. The respiratory rate increased during the infusion. Coincident with the increased respiratory rate, the flow rate of the lymph increased from 0.67 to 1.57 ml. per min. The flow rate returned to control levels within 30 minutes of completion of the infusion. The total output of red blood cells in the lymph increased transiently coincident with the increase in flow rate. Thereafter the output of red blood cells stabilized at a level slightly below that of the control period and no further decrease in output occurred over the next four hours.

Dog 440 received 550 r. whole body irradiation. The thoracic duct was cannulated 11 days later and observations were begun immediately. Results are shown in Figure 2. The output of red blood cells in the lymph appeared stable over a control period of four hours. Seventeen ml. (1.44 ml. per Kg.) of lyophilized platelets then was infused over a six minute interval. The platelet level of this recipient dog would have been expected to increase to approximately 200,000 per cu. mm. if all of these lyophilized platelets had circulated. However, the circulating platelet level did not increase. The dog vomited once immediately following the infusion and the respiratory rate increased transiently. The flow rate of the lymph increased from 0.47 to 1.22 ml. per minute and then returned to control levels in approxi-

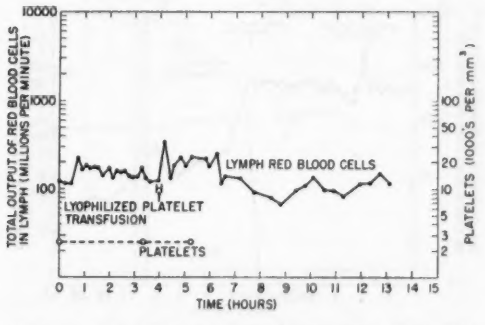

FIG. 2. 550 r. WHOLE BODY IRRADIATION (DOG 440)

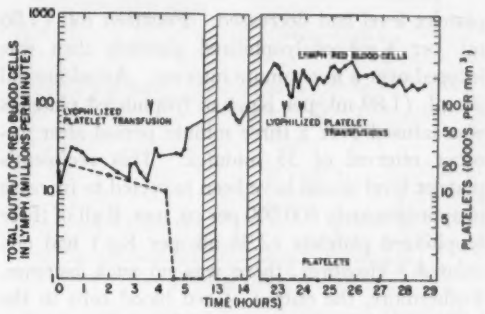

FIG. 3. 500 r. WHOLE BODY IRRADIATION (DOG 441)

mately two hours. The total output of red blood cells in the lymph increased transiently, coincident with the increase in flow rate. Thereafter the output of red blood cells stabilized at a level approximately the same as that of the control period. There was no further fall in the output over the next seven hours.

Dog 441 was given 500 r. whole body irradiation. Nineteen days later the peripheral blood platelet count was < 5,000 per cu. mm. The thoracic duct was cannulated on the twentieth post-irradiation day. Because of anemia and shock, the animal was given a transfusion of 250 ml. whole blood as the operation was being completed. This transfused blood had been collected and stored at 4° C. for seven days in a silicone coated bottle containing acid-citrate-dextrose solution. Platelet count on this blood just prior to the transfusion was 100,000 to 200,000 per cu. mm. but an accurate count was not possible because the platelets in the counting chamber were in large clumps. Fifteen minutes after this transfusion, the peripheral blood platelet count was 20,000 per cu. mm. Observations on the lymph were begun as soon as the operation was completed and the results are presented in Figure 3. The output of red blood cells in the lymph was lower than in the two previous experiments but appeared stable over a three and a half hour control period. Nineteen ml. (1.44 ml. per Kg.) of lyophilized platelets then was infused over a five minute interval. There was no increase in the circulating platelet level following this infusion, and the output of red blood cells in the lymph did not decrease. The output of red blood cells in the lymph had increased markedly 22.5 hours after operation, and the circulating

platelet level had decreased. Fourteen ml. (1.06 ml. per Kg.) of lyophilized platelets then was infused over a five minute interval. An additional 25 ml. (1.89 ml. per Kg.) of lyophilized platelets was infused over a three minute period after another interval of 35 minutes. This recipient's platelet level would have been expected to increase to approximately 400,000 per cu. mm. if all of these lyophilized platelets (2.95 ml. per Kg.) had circulated. However, there was no such increase. Futhermore, the output of red blood cells in the lymph did not decrease. The flow rate of the lymph also did not change appreciably.

Effects of infusion of lyophilized platelets followed by infusion of fresh platelets

Four dogs (426, 428, 435 and 442) received infusions of lyophilized platelets after which fresh platelets were administered.

Dog 426 received 500 r. whole body irradiation. Ten days later a platelet count was 10,000 per cu. mm. The thoracic duct was cannulated on the eleventh postirradiation day. At this time the lymph appeared milky and was shunted back into the venous cannula. On the twelfth postirradiation day the dog was still eating and appeared in reasonably good condition. The peripheral blood platelet level was < 5,000 per cu. mm., and the total output of red blood cells in the lymph was only 29.3 million per minute. Thus the observations on the lymph were interrupted, and the lymph again was shunted into the venous circulation. On the thirteenth postirradiation day, the output of red blood cells in the lymph had increased somewhat. The peripheral blood revealed a platelet count of < 5,000 per cu. mm., a whole blood clotting time of 60 minutes, a plasma fibrinogen of 363 mg. per cent and markedly impaired clot retraction. Twenty-five ml. (1.25 ml. per Kg.) of lyophilized platelets was infused over a ten minute interval. Forty-five minutes after this infusion, the peripheral platelet level remained < 5,000 per cu. mm., the plasma fibrinogen was unchanged and was determined to be 362 mg. per cent, and clot retraction remained markedly impaired, but the whole blood clotting time had returned to normal and was determined to be 19 minutes. Observations on the lymph, however, were unreliable, because the dog was not anesthetized during this infusion. Although no overt reaction to the infusion was apparent except for a transient increase in respiratory rate, the dog became very restless and active on several occasions and the flow rate of the lymph changed frequently and markedly. Observations on the lymph again were interrupted and the lymph shunted into the venous circulation. The output of red blood cells in the lymph increased strikingly by the fourteenth postirradiation day. The dog was anesthetized and continuous observations on the lymph were begun. Results are shown in Figure 4. After a control period of one and three-quarter hours, 25 ml. (1.25 ml. per Kg.) of lyophilized platelets was infused over a 13 minute interval. No increase in the level of circulating platelets followed this infusion. There was a transitory increase in total output of red blood cells coincident with a transitory increase in flow rate of the lymph. The output of red blood cells then stabilized at a level approximately the same as that during the control period. Four and one-half hours later, 37 ml. (1.85 ml. per Kg.) of freshly separated and concentrated platelets was infused over a four minute interval. This platelet suspension contained 10,000,000 platelets per cu. mm. The recipient's platelet level would have been expected to increase to a level of approximately 270,000 per cu. mm. if all of these platelets had continued to circulate. The level actually increased from < 5,000 to 145,000 per cu. mm. following this infusion, and a prompt and striking decrease in the total output of red blood cells in the lymph occurred. There was no untoward reaction to this infusion. The lymph clotted in the cannula for the first time in this experiment one hour after the infusion. The can-

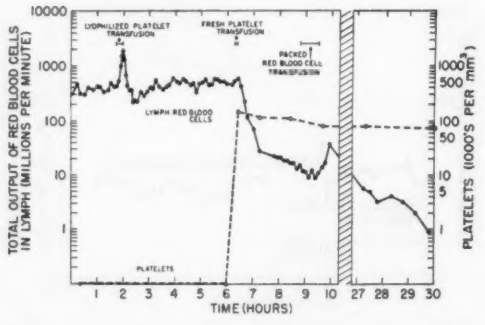

FIG. 4. 500 r. WHOLE BODY IRRADIATION (DOG 426)

nula was flushed with physiological saline solution and the lymph flow re-established at its previous rate. The peripheral blood hematocrit was 23.5 per cent, and 2.5 hours after the infusion of fresh platelets, a transfusion of 400 ml. of packed red blood cells was given. These packed red blood cells had been obtained during the differential centrifugation procedures for the preparation of the fresh platelets. The peripheral blood hematocrit was raised to 31 per cent following this transfusion. The output of red blood cells in the lymph continued to fall over the next 21 hours and the circulating platelet level remained $> 75{,}000$ per cu. mm.

Dog 428 was given 500 r. whole body irradiation. Ten days later the thoracic duct was cannulated, and the lymph appeared white with a faint pink tint. The output of red blood cells in the lymph had increased by the eleventh postirradiation day. The peripheral blood at this time revealed a hematocrit of 17 per cent and a platelet count of $< 5{,}000$ per cu. mm. The dog appeared moribund and observations on the lymph were begun. Results are shown in Figure 5. After a control period of 30 minutes, 16.4 ml. (1.06 ml. per Kg.) of lyophilized platelets was infused over a 10 minute interval. The circulating platelet level did not increase after this infusion. The output of red blood cells in the lymph increased transiently, coincident with a transient increase in flow rate. Three hours later, 390 ml. of fresh platelet-rich plasma containing 250,000 platelets per cu. mm. was infused over a 40 minute interval. The recipient's platelet count was 32,000 per cu. mm. immediately following this infusion. Five

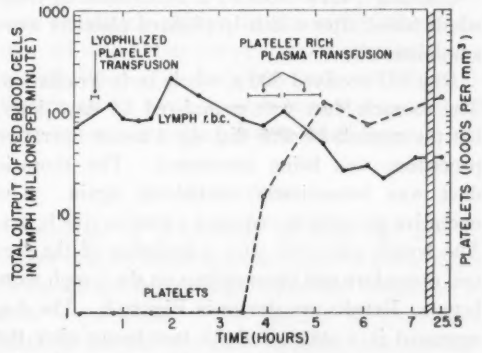

FIG. 5. 500 r. WHOLE BODY IRRADIATION (DOG 428)

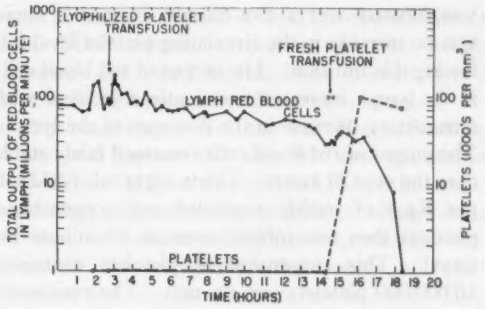
1000
100
10
LYOPHILIZED PLATELET TRANSFUSION
FRESH PLATELET TRANSFUSION
LYMPH RED BLOOD CELLS
PLATELETS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
TIME (HOURS)
TOTAL OUTPUT OF RED BLOOD CELLS IN LYMPH (MILLIONS PER MINUTE)
PLATELETS (1000'S PER mm³)

FIG. 6. 550 r. WHOLE BODY IRRADIATION (DOG 435)

minutes later, 450 ml. of fresh platelet-rich plasma containing 330,000 platelets per cu. mm. was infused over a 45 minute interval. The recipient's platelet level increased to 125,000 per cu. mm. following this infusion. The output of red blood cells gradually fell during the next two hours despite a transitory increase in flow rate of the lymph. The circulating platelet level was 140,000 per cu. mm. 20 hours after the last infusion and the output of red blood cells did not decrease further. Fresh platelet-rich plasma was used in this experiment in place of concentrated platelets because the dog probably would not have survived long enough for procedures of separation and concentration of the platelets.

Dog 435 received 550 r. whole body irradiation. Ten days later the peripheral blood platelet count was 35,000 per cu. mm. and the thoracic duct was cannulated. The lymph was not grossly bloody at the completion of the operation and the total output of red blood cells in the lymph was $< 10{,}000{,}000$ per minute. The lymph therefore was shunted into the venous circulation. The platelet count was $< 5{,}000$ per cu. mm. on the eleventh postirradiation day and the output of red blood cells in the lymph was approximately 12,000,000 per minute. The dog remained in fair general condition; observations again were interrupted and the lymph diverted into the venous circulation. The output of red blood cells had increased strikingly by the twelfth postirradiation day, and continuous observations on the lymph were begun. Results are shown in Figure 6. The output of red blood cells remained fairly stable over a two and three-quarter hour control period. Twenty-six ml. (1.58 ml. per Kg.) of lyophilized platelets then

was infused over a five minute interval. There was no increase in the circulating platelet level following this infusion. The output of red blood cells in the lymph increased transiently coincident with a transitory increase in the flow rate of the lymph. The output of red blood cells remained fairly stable over the next 11 hours. Thirty-eight ml. (2.32 ml. per Kg.) of freshly separated and concentrated platelets then was infused over an 18 minute interval. This suspension of platelets contained 10,000,000 platelets per cu. mm. The recipient's platelet level would have been expected to increase to approximately 330,000 per cu. mm. if all of these platelets had circulated. Following the infusion, the recipient's platelet level actually increased from < 5,000 to 100,000 per cu. mm., and there was a prompt and striking decrease in the total output of red blood cells in the lymph.

Dog 442 was given 550 r. whole body irradiation. The thoracic duct was cannulated 12 days later, and continuous observations of the lymph were begun. Results are shown in Figure 7. The total output of red blood cells in the lymph was very high and fairly stable during a one and one-half hour control period. Twenty-one and one-half ml. (1.43 ml. per Kg.) of lyophilized platelets then was infused over a 14 minute interval. No increase in the level of circulating platelets followed. The output of red blood cells increased transiently, coincident with a transitory increase in flow rate of the lymph. Thereafter the output stabilized at a level approximating the control level. Ten and one-half ml. (0.70 ml. per Kg.) of freshly separated and concentrated platelets was infused over a 12 minute interval six and three-quarter hours later. This suspension contained 9,000,000 platelets per cu. mm. If all of these platelets circulated, the recipient's platelet level would have been expected to increase to a level of approximately 90,000 per cu. mm. Actually the recipient's platelets increased from < 5,000 to 40,000 per cu. mm., and the output of red blood cells in the lymph decreased promptly and strikingly. The lymph flow rate did not change significantly and no untoward reaction to this infusion was observed. Seven hours later the peripheral blood hematocrit was 23 per cent. Then 500 ml. of fresh whole blood containing 230,000 platelets per cu. mm. was transfused over a 90 minute interval. This blood had been collected immediately prior to use in a nonsiliconed glass bottle containing acid-citrate-dextrose. The tubing of the donor and recipient sets was plastic. The recipient's platelet level increased from 20,000 to 85,000 per cu. mm. following this transfusion. The recipient was flushed and shivered during the transfusion. At this time the flow rate of the lymph and the output of red blood cells in the lymph increased transiently. Thereafter the output of red blood cells continued to fall. Five hours later 20 ml. (1.33 ml. per Kg.) of lyophilized platelets was administered over a seven minute interval. There was no striking change in the circulating platelet level following this infusion. The output of red blood cells increased transiently coincident with a transitory increase in the flow rate of the lymph. Over the next seven hours, the output of red blood cells in the lymph remained fairly stable.

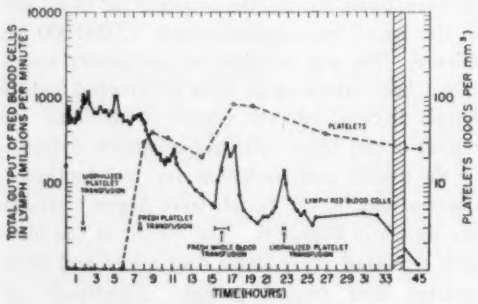

FIG. 7. 550 r. WHOLE BODY IRRADIATION (DOG 442)

Effects of infusion of fresh platelets followed by infusion of lyophilized platelets

One dog (431) received a transfusion of fresh whole blood after which lyophilized platelets were administered.

Dog 431 received 500 r. whole body irradiation. The thoracic duct was cannulated 13 days later, but the cannula became dislodged as the operative procedure was being completed. The thoracic duct was immediately cannulated again. The operative procedures required a total of five hours. The lymph was pink after completion of the second procedure and observations on the lymph were begun. Results are shown in Figure 8. The dog appeared in a state of shock two hours after the operations. An infusion of 400 ml. of platelet

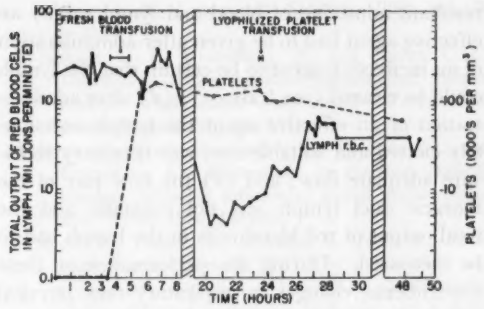

FIG. 8. 500 r. WHOLE BODY IRRADIATION (DOG 431)

poor plasma was given over a 60 minute interval. This plasma had been obtained during the differential centrifugation for the preparation of lyophilized platelets and had been stored at 4° C. for 25 days. The recipient's platelet count did not increase following this infusion and no striking change in the output of red blood cells in the lymph occurred. Two hours later a transfusion of 500 ml. of fresh whole blood was administered over a one and one-quarter hour interval. This blood was collected immediately prior to use in an uncoated glass bottle containing acid-citrate-dextrose solution with the use of plastic tubing. The blood contained 400,000 platelets per cu. mm. The recipient's platelet level increased from < 5,000 to 160,000 per cu. mm. following this transfusion, and the output of red blood cells in the lymph fell strikingly. Thereafter, the output began to rise slowly. Twenty ml. (1.42 ml. per Kg.) of lyophilized platelets was infused over a five minute interval 18 hours later. The recipient's platelet level decreased slightly following this infusion and the output of red blood cells continued to increase. The circulating platelet level was 60,000 per cu. mm. 48 hours after the operations and the output of red blood cells had increased somewhat, although not to levels so high as at the start of the experiment when the platelets were lower.

DISCUSSION

Infusions of lyophilized platelets did not increase platelet levels and had no hemostatic effect in irradiation hemorrhage under the conditions of these experiments. The number of lyophilized platelets infused into each recipient was approximately the number of platelets contained in a volume of normal dog blood equal to the recipient's total blood volume. Infusions of fresh, viable platelets did increase recipient's platelet levels and striking hemostatic effects were noted.

Our results agree with those of Woods, Gamble, Furth and Bigelow (8) who first used the output of red blood cells in thoracic duct lymph in irradiated dogs to measure the hemostatic effectiveness of various substances. Their experiments emphasized the hemostatic effectiveness of intact platelets that circulated in recipient animals. They also reported, without giving experimental details, that no hemostatic effect could be demonstrated in the test system following infusion of lyophilized platelets, frozen and thawed platelets or platelets disrupted by ultrasonic or supersonic vibrations, thromboplastin or rutin.

Fliedner and associates (15), using rats, reported that fresh, viable platelets that circulated in recipients completely controlled irradiation hemorrhage. In contrast, lyophilized platelets did not control the hemorrhage and did not circulate in recipients. Gross observations of bleeding, microscopic study of lymph node sections for evidence of bleeding, platelet counts, hematocrit values and susceptibility to bleeding from minor trauma were used to gauge the hemostatic effectiveness of the test substances in these studies.

Klein and associates have reported hemostatic benefit following infusions of platelets preserved by freezing (4) and by lyophilization (5) as judged by cessation of clinical bleeding in thrombocytopenic patients. The hemostatic effects were noted even though the recipient's circulating platelet levels did not increase following the infusions of the preserved platelets. Other platelet preparations and platelet substitutes that do not raise recipient platelet levels have been reported to have hemostatic effects in thrombocytopenic recipients (16, 17).

The question of whether a particular platelet preparation, fraction, or substitute that does not increase a recipient's platelet level, exerts a hemostatic effect on bleeding due to thrombocytopenia is difficult to answer. Measurements of capillary integrity (bleeding time and capillary resistance tests) and of blood coagulation (prothrombin consumption, thromboplastin generation, clot retraction, and so forth) can be made before and after administration of the test substance. Many of

these tests are difficult to reproduce and quantify, and the degree of bleeding in thrombocytopenic individuals is not quantitatively related to results of most of these tests. Also, hemostasis is not assured even if all of these tests are brought to normal by the test substance.

One must, therefore, measure the degree or severity of bleeding in thrombocytopenic states before and after administration of a test substance in order to determine the hemostatic effectiveness of the substance. Such measurements can be made on thrombocytopenic patients or on experimental animals rendered thrombocytopenic. Studies of thrombocytopenic patients are difficult to control and to quantify because the type and severity of bleeding vary spontaneously. Adequate controls of the factors involved are more readily achieved by the use of experimental animals rendered thrombocytopenic. The comparative effectiveness of various agents (fresh and lyophilized platelets) in controlling gross and microscopic bleeding in rats rendered thrombocytopenic by whole body irradiation has been reported. The degree of bleeding in treated animals was compared with the degree of bleeding in paired controls (15). The present report describes the comparative effectiveness of fresh and lyophilized platelets in reducing the output of red blood cells in the thoracic duct lymph of irradiated, thrombocytopenic dogs.

The method described in the present report had certain advantages, including: (*a*) thrombocytopenia was regularly and predictably produced by the dose range of whole body irradiation employed; (*b*) bleeding, including an increase in output of red blood cells in the lymph, was observed in every untreated animal that survived for a sufficient period; (*c*) enumeration of red blood cells in the lymph served as a semiquantitative measure of the degree of bleeding; (*d*) agents (viable platelets) known to be effective in controlling irradiation hemorrhage were always effective; and (*e*) an animal served as its own control. Some of the disadvantages of this test system were: (*a*) the operative procedure required adequate surgical skill; (*b*) the thoracic duct cannula had to be placed within the duct itself and not in an isolated segment of the external jugular vein (this precaution was necessary in order to preclude leakage of blood from small tributary veins into the segment of external jugular vein with resultant admixing of blood and lymph); (*c*) an effective agent had to be given after administration of an ineffective agent to be certain that the lymph could be cleared (see *b* above); (*d*) after administration of an effective agent the lymph occasionally clotted and suitable care was necessary to assure adequate flow; and (*e*) the flow rate of the thoracic duct lymph was not constant, and the total output of red blood cells in the lymph had to be measured. During the performance of these experiments, changes in respiratory rate, physical activity, rate of intravenous hydration (in anesthetized animals), vomiting and defecation influenced the flow rate of the lymph.

SUMMARY

Eight dogs were rendered thrombocytopenic by the administration of 500 to 550 r. whole body irradiation. After thrombocytopenia developed, the thoracic duct of each dog was cannulated, and large numbers of red blood cells were observed in the thoracic duct lymph. The hemostatic effectiveness of fresh and lyophilized platelets in reducing the output of red blood cells in the lymph was compared.

Lyophilized dog platelets were infused into each dog in amounts calculated to increase the recipient's platelet level by approximately 200,000 per cu. mm. The circulating platelet levels of the recipients did not increase following infusions of lyophilized platelets, and the output of red blood cells in the lymph did not decrease significantly.

Five of the dogs also received infusions of freshly separated, viable platelets. Fresh platelets were administered after the administration of lyophilized platelets in four of the animals, and in one the fresh platelets were administered initially. The circulating platelet levels of the recipients increased following infusions of fresh platelets, and the output of red blood cells in the lymph decreased strikingly.

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FACTORS INFLUENCING THE EXCHANGE OF SODIUM BETWEEN PLASMA AND CEREBROSPINAL FLUID*†

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This study is concerned with the mechanism of exchange of sodium between plasma and cerebrospinal fluid (CSF). Several workers (1, 2) have reported that there is no significant difference between the concentration of sodium in plasma and CSF. Merritt and Fremont-Smith (3) reported that the average concentration of sodium in CSF, in man, exceeded the plasma concentration by about four mEq. Davson (4) has determined the distribution ratio of sodium between CSF and plasma as 0.975 in the dog, correcting for the difference in water content of these fluids. Despite the similarity in sodium concentration of these fluids and the superficial resemblance of CSF to a protein-free filtrate of plasma, intravenously administered radio-sodium reaches equilibrium in the CSF far more slowly than in the extracellular fluid (5, 6). The delay in exchange between plasma and CSF has been attributed to the existence of a blood-CSF barrier for sodium (5–7). The present experiments have been devised to note the effect of a variety of physiological and pharmacological alterations on the exchange of sodium between these fluid compartments.

METHODS

One hundred and twenty-three experiments were performed in 27 adult mongrel dogs anesthetized with intravenously administered sodium pentobarbital (30 mg. per Kg.). Additional doses of pentobarbital were given intravenously during the experiment to maintain a stable corneal reflex and respiratory rate. Sixty to 80 microcuries of Na^{24} was administered intravenously. Samples of serum and 0.6 ml. samples of cisternal fluid were removed successively at the end of five or six 45 minute intervals. The time necessary for the activity of the cisternal fluid to reach 50 per cent of the serum activity was obtained by graphic analysis of the CSF/serum ratios at these time intervals. As the serum radioactivity was not maintained at a constant level, a time constant was not calculated but the half-time as determined was used as the measure of the rate of sodium exchange. This half-time is referred to as the sodium exchange time. A typical experiment is illustrated in Figure 1. Radioactivity was measured in duplicate with a well-type scintillation counter and sodium content with a flame photometer. Each animal had at least one control study in addition to one or more experimental studies. Each animal was given at least a seven day recovery period between experiments. Cisternal fluid pressure, when required, was measured with a spinal water manometer. Blood pressure was recorded in some experiments with a strain-gage manometer.

RESULTS

I. Concentration of sodium in serum and cerebrospinal fluid of normal dogs

The serum and CSF sodium concentrations were determined in 27 normal dogs. The average serum sodium concentration was 155 ± 6 mEq. per L. The average CSF sodium concentration was 158 ± 6 mEq. per L. Statistical analysis using Student's "t" test (8) for correlated means revealed no significant difference between these values ($p > 0.2$). The serum and CSF sodium concentrations were not significantly altered in any of the experiments. The serum sodium varied between 142 and 166 mEq. per L. and the CSF sodium between 144 and 168 mEq. per L. The difference in concentration of sodium in the CSF varied between + 9 and − 4 mEq. per L. as compared to the serum. The concentrations may be expressed as corrected for the differences in water content of serum and CSF. Assuming the water content of serum to be 92 per cent, and of CSF to be 99 per cent (7), the average concentration in plasma is 168 mEq. per Kg. of water,

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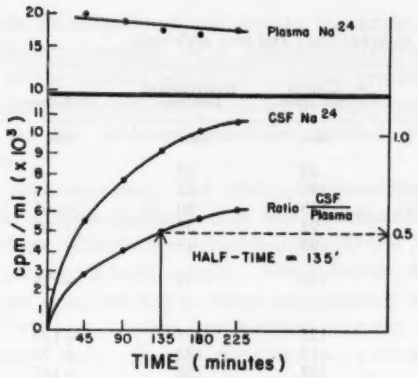

FIG. 1. CONTROL STUDY: DOG BO,

A typical experiment illustrating the determination of sodium exchange time in a normal control study.

and that of CSF is 159 mEq. per Kg. of water. The assumed distribution ratio would be 0.95.

II. Sodium exchange time in normal dogs

The average sodium exchange time in 27 normal dogs was 143 ± 38 minutes (S.D.), with a range of 90 to 260 minutes. The control study was repeated in 10 dogs at least one week after the initial study. The data are summarized in Table I. The mean difference between the paired control studies was + 3.0 ± 17.0 minutes. The mean difference of + 3.0 was found to be not significantly different statistically from zero at the .01 level of significance, by Student's "t" test (8). In subsequent experiments, the effect of the various drugs and procedures on the sodium exchange time was tested and analyzed statistically by Wald's method of sequential analysis (9). The standard deviation of differences in half-time during the control and experimental studies was assumed to be twice as large as the standard deviation of differences in half-time on duplicate control readings. The upper limit of the 90 per cent confidence interval for the true standard deviation of differences in half-time on duplicate control readings was taken as the estimate of the true standard deviation. The data are summarized in Table II.

TABLE I

Sodium exchange time in normal dogs: Duplicate studies

Dog	First study	Second study	Difference
	min.	*min.*	*min.*
BO	135	160	+25
CU	260	275	+15
UG	115	85	−30
NO	200	210	+10
XE	125	125	0
NA	110	130	+20
RO	95	95	0
WA	130	125	− 5
AT	140	125	−15
FL	140	150	+10
		Mean difference	+3.0 min.
		S.D.	±17.0 min.

III. Factors altering sodium exchange time (see Table II)

1. Vasopressin. The effect of vasopressin on the sodium exchange time was determined in six dogs. Four dogs were given intravenously 2 to 5 units of vasopressin per Kg. of body weight. Two dogs were given intravenously 5 units per Kg. of body weight and each an additional 3 units and 5 units per Kg. by intramuscular route. The intramuscular doses were divided so that 1 unit and 1.7 units respectively were given at the end of the first, second and third 45 minute intervals. A dosage of 2 units per Kg. of body weight did not alter the control time. Dosages of 5 units per Kg. of body weight resulted in a statistically significant decrease in sodium exchange time. No statistical statement can be made about the animals receiving a total of 8 and 10 units per Kg., respectively, but the data are interpreted to illustrate also an effect at the higher dosage levels. The difference between the control and experimental half-times in the five animals receiving the higher dosages varied between a decrement of 45 and 75 minutes. The fall in half-time varied between 35 per cent and 85 per cent of the control values in these animals.

Such large dosages of vasopressin have a significant effect on the blood pressure. Vasopressin, 5 units per Kg. of body weight, given intravenously, results in an acute elevation of the mean arterial blood pressure of 50 to 74 per cent above its resting level which is sustained for about 10 minutes. It gradually drops to its initial value at the end of one hour. The CSF has a parallel increase and subsequent fall in pressure. To ascertain whether the vasopressin effect on

TABLE II

Summary of control and experimental sodium exchange times and their differences

Experiment	Dog	Control half-time	Experimental half-time	Difference
		min.	*min.*	*min.*
Vasopressin				
2 units/Kg. i.v.	RO	95	95	0
5 units/Kg.* i.v.	WA†	130	85	− 45
5 units/Kg. i.v.	CL	155	90	− 65
5 units/Kg. i.v.	OL	95	35	− 60
8 units/Kg. (5 units/Kg. i.v.) (3 units/Kg. i.m.)	RO	95	45	− 50
10 units/Kg. (5 units/Kg. i.v.) (5 units/Kg. i.m.)	FA	130	55	− 75
Acetazolamide				
30 mg./Kg.	XE	125	245	+120
50 mg./Kg.	UG	115	185	+ 70
100 mg./Kg.	BO	135	250	+115
100 mg./Kg.	OL	95	225	+130
120 mg./Kg.*	FA	130	220	+ 90
Mass lesion*	PL	150	60	− 90
	CH†	175	120	− 55
CSF drainage*	CH	175	90	− 85
	FO	135	110	− 25
50% sucrose				
1.5 ml./Kg.	XE	125	140	+ 15
3.0 ml./Kg.	BO	135	135	0
3.0 ml./Kg.	XE	125	150	+ 25
5.0 ml./Kg.	FU	160	148	− 12
7.0 ml./Kg.	XE	125	135	+ 10
5% dextrose/water				
50 ml./Kg.	CL	155	125	− 30
50 ml./Kg.	XE	125	125	0
50 ml./Kg.	WA	130	150	+ 20
Steroids				
DCA				
2 mg./Kg. (2, in 20 hrs.)	BL	145	145	0
2 mg./Kg. (daily, 11 days)	BL	145	140	− 5
Cortisone				
7.5 mg./Kg. (2, in 20 hrs.)	WO	170	165	− 5
7.5 mg./Kg. (daily, 14 days)	BL	145	160	+ 15
ACTH				
2 units/Kg. (daily, 12 days)	TA	215	235	+ 20
10% carbon dioxide				
0.5 L./Kg./min.	TA	215	225	+ 10
0.5 L./Kg./min.	FA	130	155	+ 25
0.5 L./Kg./min.	CL	155	140	− 15
Norepinephrine				
0.25 mg./Kg.	NA	110	100	− 10
0.25 mg./Kg.	CE	200	210	+ 10
0.25 mg./Kg.	RO	95	95	0
0.25 mg./Kg.	OL	95	105	+ 10
0.50 mg./Kg.	NA	110	105	− 5
Insulin				
3 units/Kg.	CU	260	250	− 10
10 units/Kg.	WA	130	130	0
15 units/Kg.	XE	125	145	+ 20
Diphenylhydantoin				
20 mg./Kg.	MT	130	135	+ 5
40 mg./Kg.	WH	140	135	− 5
20 mg./Kg. (3 days)	MO	140	145	+ 5
Chlorothiazide				
100 mg./Kg.	FA	130	130	0
200 mg./Kg.	OL	95	95	0
Meralluride				
0.25 ml./Kg.	XE	125	135	+ 10
0.4 ml./Kg.	UG	115	125	+ 10
Neostigmine				
0.1 mg./Kg.	UG	115	95	− 20
0.1 mg./Kg.	OL	95	100	+ 5

* For each starred experimental group, a statistically significant change in half-time, on the average, was determined using Wald's method of sequential analysis (8). The level of significance, $\alpha 1$, was .05. The statistical tests were formulated so that a true mean sodium exchange half-time of 30 minutes (or more) would be detected with 95 per cent (or more) certainty. No statistical decision could be made about the remainder of factors.

† These dogs were not suitable for statistical analysis.

sodium exchange time was due to the action of antidiuretic hormone or a nonspecific response due to the marked elevation in blood pressure, the effect of hypertension induced by norepinephrine on the exchange time was studied (see below).

2. Acetazolamide. The effect of acetazolamide on the sodium exchange time was determined in five dogs. The dosage varied between 30 and 120 mg. per Kg. of body weight. Acetazolamide, 100 mg. per cent, was administered intravenously and the Na^{24} was injected five minutes later for the exchange study. In each case there was a marked increase in the sodium exchange time. The difference between the control and experimental half-times ranged between 70 and 130 minutes, or 54 to 137 per cent greater than the control value. The average delay in the five dogs was 98 minutes. A formal statistical decision could be made with regard to the 120 mg. dosage only, but the data are interpreted to illustrate an effect at all dosage levels administered. There was no obvious correlation, however, between the dosage administered and the magnitude of the delay. The decrease in sodium exchange time cannot be correlated with a fall in the intracranial pressure. To the contrary, the intravenous administration of acetazolamide to anesthetized dogs resulted in an increase in CSF pressure. The effect of acetazolamide, 50 mg. and 100 mg. per Kg. of body weight, 5.0 and 9.5 ml., on the CSF pressure was determined in two anesthetized dogs. The cisternal fluid pressure was recorded for a 40 minute control period prior to the injection and for 160 minutes afterwards. The data are seen in Figure 2. The intracranial pressure in a control dog was also recorded. The intravenous administration of acetazolamide in these doses resulted in a rapid increase in intracranial pressure which was sustained over the 160 minute period. An injection of 10 ml. of normal saline to the control dog was without significant effect.

3. Intracranial mass lesion. A sustained increase in intracranial pressure lasting two to three weeks may be achieved by the placement of an epidural mass of bone wax and dental cement. The methods and resulting changes have been previously described in detail (10). Similar mass lesions were created in two dogs, each having had a control study one week prior to operation. Five

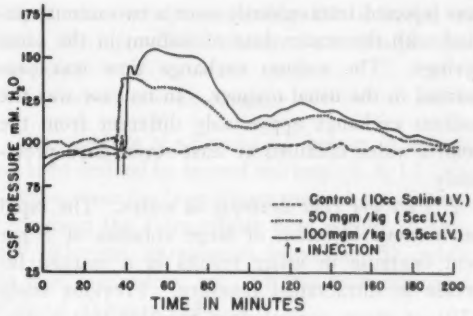

FIG. 2. EFFECT OF INTRAVENOUS ACETAZOLAMIDE ON CSF PRESSURE

days postoperatively the radio-sodium studies were repeated. The initial pressures preoperatively in each were 90 and 110 mm. water. The postoperative initial pressures were 260 and 240 mm. of water, respectively. In Dog PL the control exchange time was 150 minutes, the postoperative time was 60 minutes. In Dog CH the control exchange time was 175 minutes, the postoperative time was 120 minutes.

4. Cerebrospinal fluid drainage. The effect of a marked decrease in intracranial pressure and CSF volume on the sodium exchange time was studied by the removal (gravity and gentle syringe drainage) of all the CSF that could be obtained over a five minute period. The initial pressures in two dogs were 80 and 85 mm. water. A total of 7 ml. of CSF was removed from each animal. This usually results in an intracranial pressure of -50 to -70 mm. water (11). The tracer was then injected intravenously and the sodium exchange time determined in the usual manner. The control exchange time in the first dog was 175 minutes and in the second, 135 minutes. Following CSF drainage, the times were 90 and 110 minutes respectively.

IV. Factors not altering sodium exchange time (see Table II)

1. Fifty per cent sucrose. The rapid intravenous administration of 50 per cent sucrose results in a rapid fall in intracranial pressure (12). The effect of a hypertonic sucrose load on the sodium exchange time was determined as follows: Five experiments were performed in which 1.5 to 7.0 ml. of 50 per cent sucrose per Kg. of body weight

was injected intravenously over a two minute period with the tracer dose of sodium in the same syringe. The sodium exchange time was performed in the usual manner. In no case was the sodium exchange appreciably different from the control value obtained at least seven days previously.

2. Five per cent dextrose in water. The rapid intravenous injection of large volumes of 5 per cent dextrose in water results in a marked increase in intracranial pressure. Previous study (10) of seven normal dogs revealed that a dosage of 50 ml. per Kg. of body weight of 5 per cent dextrose in water, injected over a five minute period, resulted in a sustained increase in intracranial pressure. The average intracranial pressure was 77 per cent above the initial pressure over the first two hours and was still elevated at the end of four hours. The effect of such a water load upon sodium exchange was measured in three animals, each having had a previous control study. In each case, 50 ml. per Kg. of body weight of 5 per cent dextrose in water was given intravenously over a five minute period. Immediately thereafter Na^{24} was given and the exchange determined in the usual way. In no case was the sodium exchange rate appreciably different from the control rate obtained at least seven days previously.

3. Steroids. The effect of desoxycorticosterone acetate (DCA), cortisone and adrenocorticotropic hormone (ACTH) on the sodium exchange rate was also studied. In each dog a control study had been made at least one week earlier. The effect of DCA was studied after acute and chronic administration. In Dog BL 2 mg. of DCA per Kg. of body weight was administered intramuscularly 20 hours and again 90 minutes prior to beginning the Na^{24} study. The control sodium exchange time was 145 minutes and the experimental time was 145 minutes revealing that this dosage of DCA did not alter sodium exchange. This dog was subsequently given DCA daily, 2 mg. per Kg. of body weight intramuscularly for 11 days. Then the sodium exchange study was repeated, revealing a half-time of 140 minutes. Thus neither acute nor chronic administration of DCA appreciably altered the sodium exchange time. The prolonged administration of DCA did not appreciably alter the serum or CSF sodium concentration or the CSF potassium concentration. The serum potassium, however, fell from a control value of 4.1 mEq. per L. to 2.7 mEq. per L.

The effect of cortisone was studied after acute and chronic administration. In Dog WO 7.5 mg. of cortisone per Kg. of body weight was given intramuscularly 20 hours and again 90 minutes prior to beginning the Na^{24} study. The control sodium exchange time was 170 minutes and the experimental time was 165 minutes, revealing that this dosage of cortisone did not alter sodium exchange. Another dog, BL, was given cortisone at the rate of 7.5 mg. per Kg. of body weight daily for 14 days. The control exchange time in this animal was 150 minutes. Following the chronic cortisone administration, the half-time was 160 minutes. Thus neither acute nor chronic administration of cortisone appreciably altered the sodium exchange time.

The sodium exchange time was studied in one dog following intramuscular administration for 12 days of ACTH, 2 units per Kg. of body weight per day. The control sodium exchange time was 215 minutes. The exchange time after ACTH was 235 minutes. Thus no appreciable change in the sodium exchange time was detected.

4. Ten per cent carbon dioxide in oxygen. The effect of inhalation of 10 per cent carbon dioxide in oxygen on the sodium exchange time was studied in three dogs. A cuffed endotracheal tube, 24 inches long, was inserted to the carina and the gas mixture supplied by a catheter inserted in the endotracheal tube to reach the carina. The gas mixture was supplied at a rate of 0.5 L. per Kg. of body weight per minute for 10 minutes, then the tracer dosage was given, and the gas mixture was continued for the following 225 minutes. The sodium exchange time was not appreciably affected in any case.

5. Norepinephrine. The effect of norepinephrine was studied in five dogs. These animals were given very large doses to maintain a mean blood pressure level elevation between 50 and 150 per cent above the control pressure. These acute systemic blood pressure elevations were paralleled by an elevation in CSF pressure. Norepinephrine bitartrate was given (2 mg. norepinephrine bitartrate is equivalent to 1 mg. of norepinephrine base) by the intramuscular route, in

dosages of 0.25 mg. or 0.5 mg. per Kg. of body weight. Half the dose was given at zero time and one-quarter the dose at one hour and at two hours after the injection of the Na^{24}. In no case was the exchange time appreciably altered by the sustained hypertension.

6. Insulin. The effect of large dosages of insulin on the sodium exchange time was determined in three dogs. Each dog received 3, 10 or 15 units of crystalline insulin per Kg. of body weight. The dogs were fasted for at least 12 hours prior to these experiments. The insulin was given intravenously 10 minutes before the injection of the Na^{24} for determination of the exchange time. In each case, there was no appreciable change in sodium exchange time.

7. Diphenylhydantoin. The effect of parenteral administration of diphenylhydantoin was determined in three dogs. In two dogs diphenylhydantoin, 20 mg. and 40 mg. per Kg. of body weight, was injected intravenously five minutes prior to the injection of Na^{24} for the exchange study. In neither animal did the sodium exchange time differ significantly from the control value. In one dog diphenylhydantoin, 20 mg. per Kg. of body weight, was injected intramuscularly for three days. Twenty-four hours after the third injection the sodium exchange study was repeated. The half-time did not appreciably differ from the control value previously obtained.

8. Chlorothiazide. The effect of chlorothiazide on the sodium exchange time was determined in two dogs. The animals were given 100 mg. and 200 mg. chlorothiazide intravenously 10 minutes prior to the injection of Na^{24} for the exchange study. In neither animal did the experimental half-time differ from the control value.

9. Meralluride. The effect of meralluride (Mercuhydrin®) on the sodium exchange time was determined in two dogs. The dogs received meralluride by the intramuscular route 18 hours and 90 minutes prior to injection of Na^{24}. The dogs were given 0.25 ml. and 0.4 ml. per Kg. of body weight at each of these times. In neither case did the experimental half-time differ appreciably from the control value.

10. Neostigmine. The effect of neostigmine on the sodium exchange time was determined in two dogs. The dogs were both given intravenously 0.1 mg. per Kg. of body weight of neostigmine methyl sulfate immediately preceeding the injection of Na^{24}. No appreciable change in exchange time was noted.

DISCUSSION

The existence of a blood-CSF barrier to sodium has been verified by several workers (5, 6, 13, 14). Radio-sodium has been shown to reach equilibrium throughout the extracellular fluid within 25 minutes following intravenous injection (5), while the equilibrium time for CSF has been estimated at about 20 hours (13).

No statistical difference between the concentration of sodium in serum and CSF was detected, using the flame photometer, confirming previous reports in man (1) and dogs (2). However, a distribution ratio (CSF/serum) of 0.95 was calculated, correcting for differences in water content of the fluids. Davson (4) has reported differences in the distribution ratio in various species. It varied between 0.97 in a horse and 1.04 in guinea pigs, and was 0.975 in dogs. This interspecies variation has not been explained.

The rate of exchange of Na^{24} between the plasma and CSF is probably dependent on many independently operating permeability factors. The "barrier" anatomically must be considered to be composed of a number of structures, including the capillary endothelium, pia-glial membranes, choroid plexus, ependyma and arachnoid membrane, each of which probably has different permeability characteristics. The methods utilized in this study are considered to measure the net result of many factors affecting the exchange rate, and they do not throw light on the relative importance of the various membranes which constitute the barrier. The control studies have shown that the methods used have a satisfactory degree of reproducibility, though only major changes in exchange time can be detected. The marked variation of the control values in different dogs required that each dog be used as its own control. This variation is probably related to the anatomical variation among mongrel dogs. The importance of removing a standard amount of CSF at a standard time interval in determining the exchange time is demonstrated by noting the effect of spinal drainage. Thus a somewhat different exchange time would be expected if, for example, 1 ml. samples were removed at 30 minute intervals. This may par-

tially explain differences in half-times as reported by various authors (5, 6, 13).

A major limitation of the method used is the failure to take into account alterations in the volume of CSF. The exchange times in a given dog are comparable in various experimental situations only if the volume of CSF into which the tracer equilibrates remains constant. There is no satisfactory method for determining CSF volume *in vivo*—a major limiting factor in research with regard to the physiology of the cerebrospinal fluid. Thus, in the present experiments which record a marked decrease in exchange time following CSF drainage, it is impossible to state whether this represents increased permeability of the membranes secondary to the pressure change or whether the permeability is unchanged but the tracer is distributed into a much smaller volume of CSF. With this consideration in mind, the various experimental procedures and the possible interpretation of the data will be considered.

The administration of vasopressin resulted in a dramatic fall in sodium exchange time. This effect was not secondary to the hypertension that large doses of vasopressin produced, as even greater blood pressure elevation obtained with norepinephrine did not alter the sodium exchange time. Ussing has demonstrated that vasopressin strikingly increases the exchange of sodium and water across the frog epithelium (15). The hormone is considered to increase the size of individual pores or cellular channels in the membrane (16). Berliner, Levinsky, Davidson and Eden (17) also consider the effect of vasopressin on urinary concentration to result from the action of the hormone on tubular epithelial permeability. The effect of vasopressin on the blood-CSF barrier may be considered analogous to its effect on frog epithelial membranes, *i.e.*, it facilitates ion and solvent exchange by increasing pore size or cellular channels in the membrane. It should be noted that this effect was obtained with amounts far exceeding the physiological range. The observation is of particular interest in that it suggests the hormone may affect mammalian membranes other than the renal epithelium. It is unlikely, however, that vasopressin has any importance physiologically in the control of the permeability of the blood-CSF barrier.

Acetazolamide, an inhibitor of the enzyme carbonic anhydrase, is present in high concentration in the choroid plexus and brain as well as in red cells and kidney (18). The present studies have revealed the sodium exchange time to be greatly prolonged by acetazolamide, confirming a previous report of Davson and Luck (14). Carbonic anhydrase presumably facilitates sodium transport in tissues by making hydrogen ions available for exchange (19). Acetazolamide does not alter the distribution ratios of sodium, potassium and chloride between CSF and plasma (18), *i.e.*, it decreases the rate of exchange of sodium but not its concentration. Thus sodium exchange between the plasma and CSF is in part dependent upon an enzyme which facilitates "active transport" of the ion. The cellular elements concerned with this secretory process probably include the choroid plexus as well as the cerebrum, both having a high concentration of the enzyme (18). In the present studies, acetazolamide administration resulted in a marked increase in CSF pressure. There is considerable divergence in the literature about the effect of acetazolamide on CSF pressure with reports of both increased (20–22) and decreased (18,23–25) CSF pressure resulting from administration of the drug. These differences depend in part upon the route of administration of the drug. The decrease in pressure, reported by Tschirgi, Frost and Taylor (24) after intravenous injection in artificially respired paralyzed cats, has been attibuted to the hypertonicity of the acetazolamide solution that was administered (26). It appears likely that intravenous administration results in an acute increase in intracranial pressure because of a local tissue increase in carbon dioxide tension which results in cerebrovascular dilatation (21). The explanation for the reported decrease in pressure with chronic oral administration in patients (18, 25) is not clear. It cannot be secondary to the decreased sodium exchange rate as the intracranial pressure may vary independently of the exchange rate, nor has there been any definite change reported in pH, carbon dioxide, bicarbonate, or state of hydration to explain these observations. It appears likely that variation in the level of the CSF pressure is largely dependent upon acute vascular adjustments. Increased pressure is avoided with oral administration of the drug because sudden local increases in carbon dioxide tension probably do not occur.

The data have demonstrated that Na^{24} exchange is more rapid in the presence of an epidural mass lesion associated with increased intracranial pressure. Since the presence of the intracranial mass results in a decreased intracranial volume, a decreased CSF volume may contribute to the apparent increase in sodium exchange. However, postmortem examination has revealed that the brain compression is marked and there is only a slight decrease in ventricular volume (10). It is likely that the increased permeability of the blood-brain barrier adjacent to mass lesions, which is reflected by the increased permeability to radio-iodinated serum albumin (27) and radio-phosphorus (28), is also partly responsible for the apparent increase in penetration of radio-sodium into the subarachnoid space. This may be considered analagous to the increase in protein content of the CSF commonly associated with mass lesions.

Major osmotic shifts did not affect the sodium exchange time. The large amounts of hypertonic sucrose administered reduced the intracranial pressure to about 50 per cent of the initial pressure (12). The rapid intravenous administration of 5 per cent dextrose in water, as given, resulted in a 77 per cent increase in intracranial pressure (10). While alterations in the volume of the CSF and of the fluid compartments of brain under these circumstances cannot be quantified, the sodium exchange appears to continue at a rate independent of the induced osmotic changes.

The effect of DCA, cortisone and ACTH on the sodium exchange time was studied in view of the effect of these compounds on brain function, notably convulsive threshold. Cortisone has been reported to lower, and desoxycorticosterone to raise, electroshock threshold in animals and man (29, 30). Woodbury, Timaris and Vernadakis have also reported that cortisone and DCA have opposing effects on sodium transport in rat brain (31). In the present study this phenomenon could not be demonstrated with regard to sodium exchange between serum and CSF. Davson has shown that radio-sodium reaches equilibrium in brain and CSF at the same rate (7), suggesting a common mechanism. The failure to obtain an effect in the present studies, similar to that described by Woodbury and associates (31), is not explained.

The administration of 10 per cent carbon dioxide in oxygen results in a marked increase in cerebral blood flow as well as a reduction in the pH of both plasma and CSF (32). The inhalation of 5 per cent carbon dioxide in oxygen has been reported to result in an increase in the rate of entry of radio-sodium into the CSF in human subjects (33). Inhalation of 50 per cent carbon dioxide has also been reported to result in an increase in brain intracellular sodium concentration in rats (34). In the present study, however, carbon dioxide inhalation did not affect the sodium exchange time.

The effect of large dosages of insulin was studied to detect whether profound hypoglycemia, with its associated change in brain function, might be reflected in a change in permeability of the blood-CSF barrier to sodium. Insulin hypoglycemia, which suppresses cerebral oxygen consumption and metabolism (35), did not affect the permeability of the barrier to sodium in the dosage used.

The effects of chlorothiazide and meralluride on sodium exchange were surveyed because both of these compounds have a profound effect on ion transport in the renal tubule, and it was considered reasonable to determine whether an effect on the blood-CSF barrier, particularly on the choroid plexus, could be detected. Chlorothiazide interferes with both sodium and chloride transport across the tubular epithelium (36), while the mercurials primarily effect chloride exchange, with the changes in sodium excretion generally considered as secondary phenomena (37). Neither of these drugs, however, had any detectable effect on sodium exchange in the present experiments.

Study of the effect of diphenylhydantoin on the sodium exchange time was prompted by Woodbury's report that diphenylhydantoin accelerates the active process controlling sodium movement across brain cells in rats (38). The present study has failed to reveal any effect of diphenylhydantoin on the sodium exchange between plasma and CSF.

Study of the effect of neostigmine on sodium exchange was prompted by the reports of Greig, Holland and Mayberry (39, 40) and Becker and Aird (41) implicating the acetylcholine-cholinesterase system in the control of cerebral permeability. The present study has failed to reveal any

effect of neostigmine on the permeability of the blood-CSF barrier to sodium.

In summary, the present studies have revealed that the exchange of sodium between serum and CSF is strikingly constant in the face of a wide variety of pharmacological and physiological alterations. Thus, the following procedures failed to alter exchange: major osmotic shifts in either direction; large doses of cortisone, DCA and ACTH; 10 per cent carbon dioxide; sustained hypertension induced by norepinephrine; insulin hypoglycemia; chlorothiazide; meralluride; and neostigmine. Sodium exchange was unchanged in the face of increased intracranial pressure induced by a load of 5 per cent dextrose in water or by norepinephrine, and was also unaffected by decreased intracranial pressure induced by a hypertonic sucrose load. Permeability to sodium was increased by extradural cerebral compression which has been considered above as a nonspecific effect. The apparent increase in exchange following drainage cannot be satisfactorily evaluated. The most striking effects have been obtained with acetazolamide and vasopressin. It is concluded that the acetazolamide effect proves that sodium exchange is in part dependent upon active transport of the ion. The vasopressin effect is interpreted as an example of the ability of antidiuretic hormone to increase membrane permeability in mammalian tissues other than the renal tubule.

These demonstrations of the relative constancy of sodium exchange supplement the previous studies of Cooper, Lechner and Bellet (2) which revealed that the ionic composition of CSF is maintained within very narrow limits despite major changes in the plasma electrolyte concentration. There is abundant evidence that the CSF is in dynamic equilibrium with the extracellular fluid of brain (7, 42). Recent evidence, largely derived with electron microscopy, throws doubt on the existence of an extracellular fluid space in brain and suggests that the extracellular fluid space represents the neuroglial cytoplasm (43). For the present, however, the extracellular fluid space, regardless of its true structural nature, is a useful concept. It appears likely that the blood-CSF barrier, anatomically a complex of membranes with selective permeability, functionally serves to stabilize the composition of the CSF and in turn the brain extracellular fluid, and so contributes to the maintenance of a stable internal environment for the central nervous system.

SUMMARY

The exchange of Na^{24} between plasma and cerebrospinal fluid (CSF) has been studied in anesthetized dogs. The average exchange time (half-time) in 27 normal dogs was 143 ± 38 minutes (S.D.). The administration of vasopressin resulted in a decrease in the exchange time. The intravenous administration of acetazolamide resulted in an increase in the exchange time and in a marked increase in intracranial pressure. Both increased intracranial pressure secondary to an epidural mass and decreased intracranial pressure secondary to CSF drainage resulted in a decrease in the exchange time. The exchange time was not affected by the following drugs and procedures: cortisone, desoxycorticosterone or corticotrophin, hypertonic or hypotonic intravenous solutions, 10 per cent carbon dioxide, norepinephrine, insulin hypoglycemia, diphenylhydantoin, chlorothiazide, meralluride or neostigmine. There was no direct correlation between change in intracranial pressure and the sodium exchange time. The significance of these data with regard to the blood-CSF barrier has been discussed.

ACKNOWLEDGMENT

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SUPRALETHAL WHOLE BODY IRRADIATION AND ISOLOGOUS MARROW TRANSPLANTATION IN MAN *†

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Infusions of normal marrow will prevent death from marrow failure in animals that have received lethal doses of total body irradiation, doses of the order of 1,000 roentgens (r.) (1). By analogy similar infusions might be expected to be useful in treating postradiative marrow failure in man. Two patients with leukemia requiring treatment by radiation have been given 850 r. and 1,140 r., respectively. Each patient had an identical twin to serve as donor of normal isologous marrow. In these twins it was possible to study the problems of lethal irradiation and marrow restoration free from the immunologic complications of homotransplantation. It was also possible to observe the effect of lethal irradiation upon leukemia in man.

METHODS

The methods of obtaining, preparing, storing and infusing bone marrow have been described previously (2, 3, 4). The two Co^{60} units used to administer whole body irradiation have also been described (5). All blood transfusions were of freshly obtained blood drawn into plastic bags containing 50 ml. of 1.5 per cent ethylenediamenetetraacetic acid (EDTA) and 0.7 per cent NaCl.[1] A platelet transfusion (one unit of platelets) represented the platelet concentrate from 500 ml. of blood obtained by differential centrifugation in plastic bags[1] (6). Platelet counts were performed with a phase microscope by the method of Brecher and Cronkite (7). Reticulocytes were counted by the method of Brecher (8). Hemoglobin was determined by the cyanmethemoglobin method (9).

CASE REPORTS

Case I: (D. C. Number 79389). This colored female aged two years and eleven months was admitted to the Mary Imogene Bassett Hospital for the first time on October 4, 1958. She was one of identical twins with a history of normal development. She was seen at the Harriet Lane Home of the Johns Hopkins Hospital, Baltimore, Maryland on June 11, 1958 because of swelling of the eyes and lips, diagnosed as angioneurotic edema, which responded to Benadryl®. She was admitted to the Harriet Lane Home on July 25, 1958 because of night sweats, vomiting, mild fatigue and a low-grade fever. At that time her white blood cell count was 45,000 per cu. mm., and the differential showed 57 per cent blast cells. The hematocrit was 20 per cent. A bone marrow study showed hypocellularity and numerous blast cells. A diagnosis of acute leukemia was made. Initially the patient was treated with 6-mercaptopurine, 2.5 mg. per day. On August 29, 1958 Prednisone® was started at 30 mg. per day and increased to 40 mg. per day on September 9. On September 26, 6-mercaptopurine was discontinued and Methotrexate® was started at 2.5 mg. per day. There was no sign of remission, and the patient's persistent anemia required transfusions of 250 ml. of whole blood on August 14, August 27 and September 14. Because of failure to secure a remission on chemotherapy and because the patient had an identical twin, it was decided to transfer her to the Mary Imogene Bassett Hospital for whole body irradiation and marrow transplantation.

Her physical examination on admission showed resemblance to Cushing syndrome. There were shotty cervical and inguinal nodes and one small left axillary node. The abdomen was protuberant, and the spleen extended 2 cm. below the left costal margin. The liver was not enlarged.

Laboratory data showed a normal urinalysis. The hemoglobin was 4.6 Gm. per cent. The white blood cell count was 3,600 per cu. mm. with 19 polymorphonuclear leukocytes, 2 band forms, 2 blast cells, 50 lymphocytes, 5 atypical lymphocytes, 1 young lymphocyte and 21 monocytes. There were 20 nucleated red cells per 100 white cells. The platelet count was 33,000 per cu. mm. and the reticulocyte count 2.7 per cent. A chest X-ray was normal.

The patient was continued on Prednisone®, 40 mg. daily. She had been receiving Methotrexate®, 7.5 mg. daily. This drug was discontinued on October 9. The patient was placed in strict isolation. She was observed for infection, and frequent cultures showed no significant organisms. Whole body irradiation was started on

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[1] Fenwal EDTA Blood-Pack, Ethicon, Somerville, N. J.

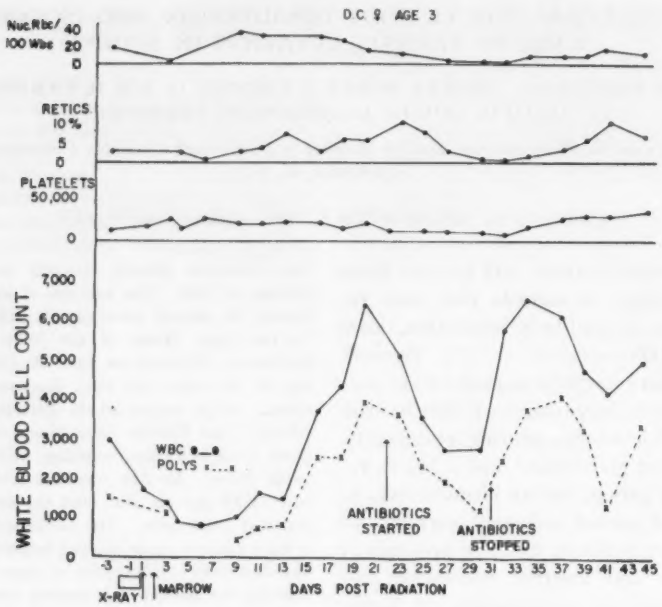

FIG. 1. HEMATOLOGIC EVENTS IN CASE 1

First irradiation. The small rectangle labeled "X-ray" indicates the administration of a tissue dose of 200 r. The arrows on the abscissa indicate marrow infusions.

October 10. Irradiation was administered with a General Electric Maximar Unit operating at 250 KV. and 5 ma., HVL 2.2 mm. Cu. On October 10 she received an air dose of 75 r. to the front of the body and also to the back of the body. The total irradiation time was 225 minutes. This procedure was repeated on October 11. The total calculated mean midline tissue dose was 200 r.

The marrow donor was considered to be an identical twin on the basis of appearance, a documented single placenta and identical blood type. On October 7 the marrow donor was taken to the operating room, and under general anesthesia 25 marrow aspirations were performed on the sternum, tibiae and anterior and posterior iliac crests. This marrow was transferred immediately to Hanks' solution containing 0.1 mg. of Connaught heparin [2] per ml. and passed through stainless steel screens. A total of 0.92×10^9 nucleated marrow cells was obtained. A differential count on this marrow showed it to be normal. The marrow was frozen in 15 per cent glycerol and stored at $-80°$ C. (4). On October 12 this marrow preparation was thawed, deglycerolized and administered to the irradiated recipient. On October 13 the marrow donor was again subjected to 20 marrow aspirations under general anesthesia from the above listed sites. A total of 0.35×10^9 nucleated marrow cells was obtained, screened and administered immediately to the recipient. There was no reaction to the administration of either of these marrow preparations.

The hematologic events postradiation are shown in Figure 1. The patient did not vomit or exhibit diarrhea as the result of the irradiation. She did quite well clinically. On the fourth day following irradiation she was given one unit of platelets. On the ninth day she was discovered to have a hemolytic staphylococcus aureus, coagulase negative in her throat culture. This organism was sensitive to chloramphenicol and erythromycin. On the twenty-second postirradiation day her temperature went up to 103.4 F°. There was no clinical evidence of infection. However, she was started on Chloramphenicol®, 800 mg. daily, erythromycin, 800 mg. daily, and Mycostatin®, 300,000 units daily. Over the next four days the patient's temperature varied from normal to 103°. Thereafter she became afebrile except for diurnal variations with maximum readings of 100.2°. On the thirty-first postirradiation day antibiotics were stopped. The changes in the white blood cell count during the period of antibiotic administration are illustrated in Figure 1. On the twenty-fifth postirradiation day the patient was given 300 ml. of fresh whole blood. A bone marrow study on that day showed a cellular marrow. Several normal megakaryocytes were seen. The myeloid-erythroid ratio was 5 to 1. There was an increased number of mature lymphocytes but no abnormal cells. This was in-

[2] Connaught Medical Research Laboratories, Toronto, Canada.

terpreted as normal marrow without evidence of leukemia.

The patient continued to feel well and her activities were increased. Between the sixteenth and the twenty-eighth postirradiation day, Prednisone® was decreased to a level of 10 mg. daily. The patient was discharged from the hospital on November 25, the forty-fifth day after irradiation.

The patient did well for approximately three weeks and then began to run a low-grade fever. She was seen at the Harriet Lane Home on December 29, at which time there was no clinical evidence of infection. She had had no bleeding. The hemoglobin was 6 Gm. per cent, the white blood cell count 6,300 per cu. mm. and the platelet count 58,000 per cu. mm. Blood smears showed 15 per cent monocytes and 4 per cent blast cells, interpreted as monoblasts. In the meantime, the twin Co^{60} irradiation unit had been placed in operation at the Mary Imogene Bassett Hospital. Accordingly, it was decided to re-irradiate the patient at a higher dose level. She was admitted to the Mary Imogene Bassett Hospital on January 17, 1959. Prednisone® had been discontinued on December 29 and the previously observed Cushing syndrome features were no longer present. There were petechiae on the buccal mucosa. The spleen was not palpable.

Initial laboratory data showed a normal urinalysis. Peripheral blood counts are given in Figure 2A. A bone marrow study before radiation showed a cellular marrow with occasional megakaryocytes. Approximately 50 per cent of the marrow cells were blasts of stem cell type.

Whole body irradiation with the Co^{60} sources was started on January 22 and completed on January 23. The irradiation time was 22 hours and 20 minutes, and the total elapsed time was 26 hours and 21 minutes. The total dose administered was 1,140 r. in air measured at the midline of the patient. (Calculated midline tissue dose, 1,003 r.).

Marrow was obtained from the normal twin as previously described. On January 24, the first day after irradiation, a total of 1.36×10^9 nucleated marrow cells was administered. On January 28, the fifth postirradiation day, marrow was again obtained from the twin and a total of 0.95×10^9 cells was administered to the patient. Because of failure of the platelet count to recover, a third series of marrow aspirations was performed on February 26, the thirty-fourth day following irradiation. This material was not screened but was administered immediately to the recipient. Approximately 0.1×10^9 nucleated marrow cells was administered directly into the posterior iliac crest, and an additional 0.8×10^9 nucleated cells was given intravenously. There was no evidence of reaction to the marrow infusions.

The postradiation hematologic data are shown in Figure 2A, and the clinical course of events is shown in Figure 2B. The patient was given oral neomycin for a period of four days before irradiation was begun. On the first day after irradiation she was started on gamma

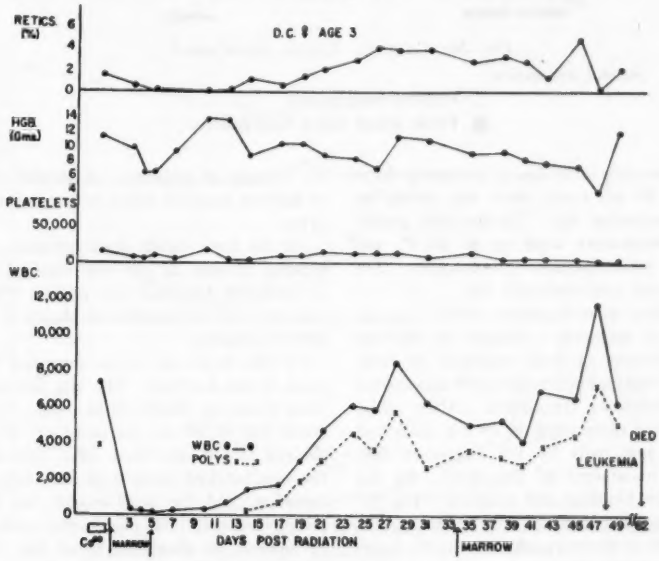

FIG. 2A. HEMATOLOGIC EVENTS IN CASE 1

Second irradiation. The small rectangle labeled "Co^{60}" indicates the administration of a midline air dose of 1,140 r. The arrows on the abscissa indicate marrow infusions.

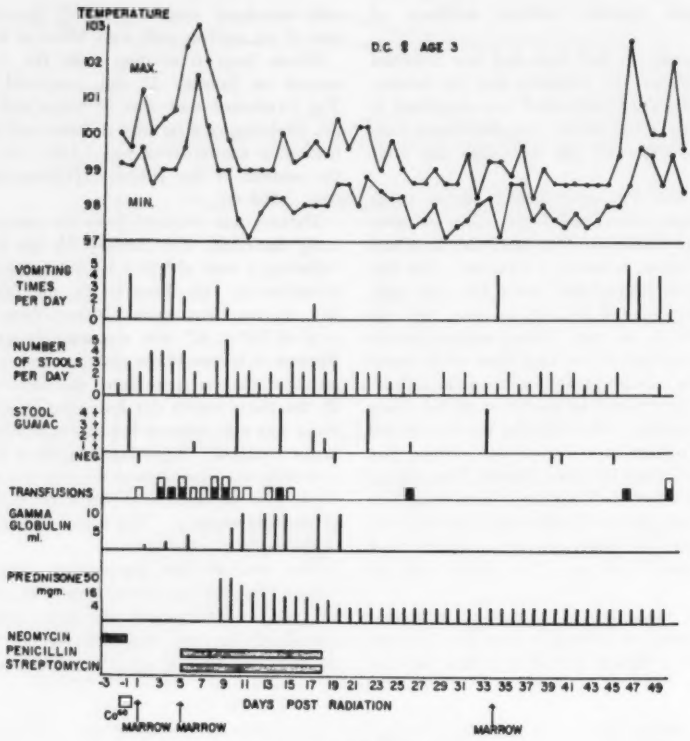

FIG. 2B. CLINICAL EVENTS IN CASE 1

Second irradiation.

□ Platelet transfusions.

■ Fresh whole blood transfusions.

globulin, 1 ml. intravenously with slowly increasing doses up to approximately 10 ml. every other day ending on the nineteenth postirradiation day. On the fifth postirradiation day her temperature went up to 102.4°, and she was started on penicillin and streptomycin. She was afebrile by the ninth postirradiation day.

During the immediate postirradiation period the patient suffered repeated epistaxes. Because of this she received frequent infusions of fresh platelets or fresh whole blood. Because of bleeding Prednisone® was started on the eighth day following irradiation. After initial doses of 50 mg. she was maintained on 16 mg. daily and slowly tapered to 4 mg. daily by the nineteenth day. Thereafter she was maintained at this level. By the eleventh day the major bleeding had subsided. The patient was eating well. She then did well clinically, except for febrile reaction to blood transfusions on the forty-sixth and forty-ninth postirradiation days.

On the thirty-second postirradiation day a marrow aspiration was performed and numerous particles were obtained. The myeloid-erythroid ratio was 5 to 1 with a normal maturation sequence in both series. There was no evidence of leukemia. A careful search of a number of marrow particles failed to demonstrate any megakaryocytes.

On the forty-eighth postirradiation day a marrow aspiration showed 22 per cent blast cells and a diagnosis of recurrent leukemia was made. The patient was discharged from the hospital on March 17, the fifty-third day after irradiation.

On March 21 she fell at home and developed a hematoma of the forehead. She was admitted to the Harriet Lane Home on March 22 in coma. The white blood cell count was 61,700 per cu. mm., and the differential count showed 35 per cent blast cells. She died on March 26, the one hundred sixty-sixth day following the first irradiation, and the sixty-second day following the second irradiation. A postmortem examination was not performed. It should be noted that the remission after 1,140 r. was 48 days whereas it was 66 days after the original 200 r.

Case II: (*D. W. Number 80151*). This white female aged four years and nine months was admitted to the Mary Imogene Bassett Hospital on December 10, 1958.

She was one of identical twins with a normal history of growth and development. In November of 1957 she complained of aches and pains. Progressive lethargy and increased irritability followed. In April of 1958 she was admitted to the Eglin Air Force Base Hospital. The hemoglobin was 6.5 Gm. per cent, and the white blood cell count was 5,200 per cu. mm. A surgical bone marrow biopsy was performed, and the diagnosis of acute leukemia was made and confirmed by the Armed Forces Institute of Pathology. She was treated with prednisolone, 20 mg. a day, and a complete remission was obtained in five weeks. She was maintained on 15 mg. prednisolone daily. In October of 1958 she again had increasing lethargy and irritability. On several occasions she had generalized convusions, at one time requiring a tracheotomy for control of oropharyngeal secretions. The spinal fluid was normal. At this time her hemoglobin was 6 Gm. per cent, and bone marrow studies showed the predominant cell to be a lymphoblast. She was treated with transfusions and Aminopterin®, 0.5 mg. per day, but the drug had to be discontinued after 10 days because of ulcerative stomatitis. On November 19, 1958 she was started on Dilantin® for control of convulsions and Methotrexate®, 2.5 mg. daily. Prednisolone was discontinued after a single dose of 40 units of corticotropin intramuscularly. After three weeks she showed no evidence of remission in her leukemia. For this reason she was transferred to the Mary Imogene Bassett Hospital for whole body irradiation and marrow transplantation.

Physical examination on admission showed scattered ecchymoses. There were a few small firm lymph nodes in the neck. The spleen was enlarged 2 cm. below the left costal margin. There was a well healed tracheotomy scar.

The hemoglobin was 15.3 Gm. per cent. The white blood cell count was 8,300 per cu. mm. with 57 polymorphonuclear leukocytes, 26 lymphocytes, 9 monocytes, 1 eosinophile, 2 band forms, 1 atypical lympocyte, 3 young lymphocytes and 1 blast cell. The platelet count was 139,000 per cu. mm. Stool was guaiac negative. On December 13 a bone marrow aspiration showed 40 to 50 per cent blast cells.

On admission to the hospital the patient was placed in strict isolation. She was continued on Dilantin® and phenobarbital, but Methotrexate® was discontinued. No steroids were given. On December 14 she was started on oral neomycin, 2 Gm. daily and Compazine®, 5 mg. daily. Whole body irradiation from the twin Co^{60} units was carried out on December 15 and 16. The duration of irradiation was 24 hours and 20 minutes. The elapsed time was 27 hours and 22 minutes. The total dose measured in air at the midline of the patient was 850 r. (calculated midline tissue dose, 748 r.). The patient had been vomiting one to three times daily and having considerable nausea before irradiation was started. This did not change during the period of irradiation but ended on the second postirradiation day. She had no significant diarrhea.

The patient's twin sister was assumed to be an identical twin on the basis of appearance and identical blood type. On December 17, the first day after irradiation, the normal twin was taken to the operating room where, under general anesthesia, a total of 20 marrow aspirations were performed on the tibiae and anterior and posterior

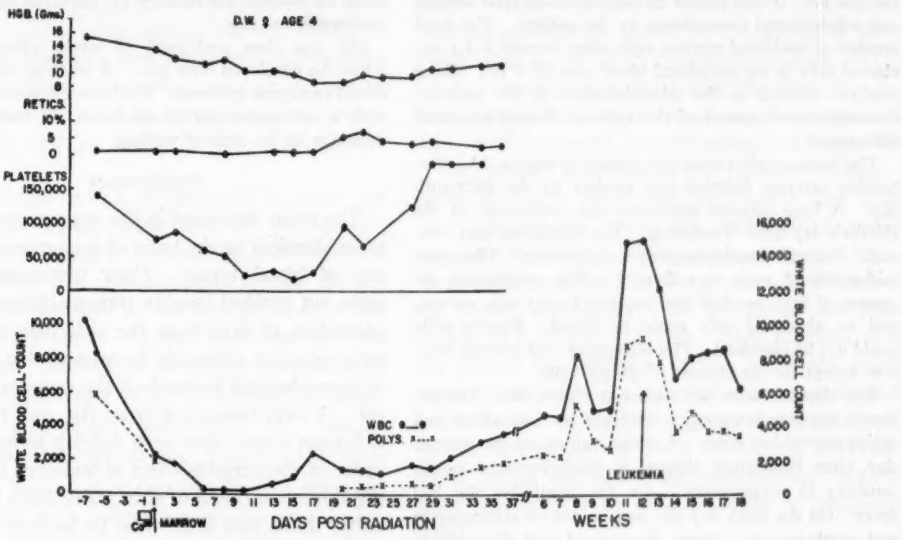

FIG. 3A. HEMATOLOGIC EVENTS IN CASE 2

The small rectangle labeled "Co^{60}" indicates the administration of a midline air dose of 850 r. The arrow on the abscissa indicates marrow infusion.

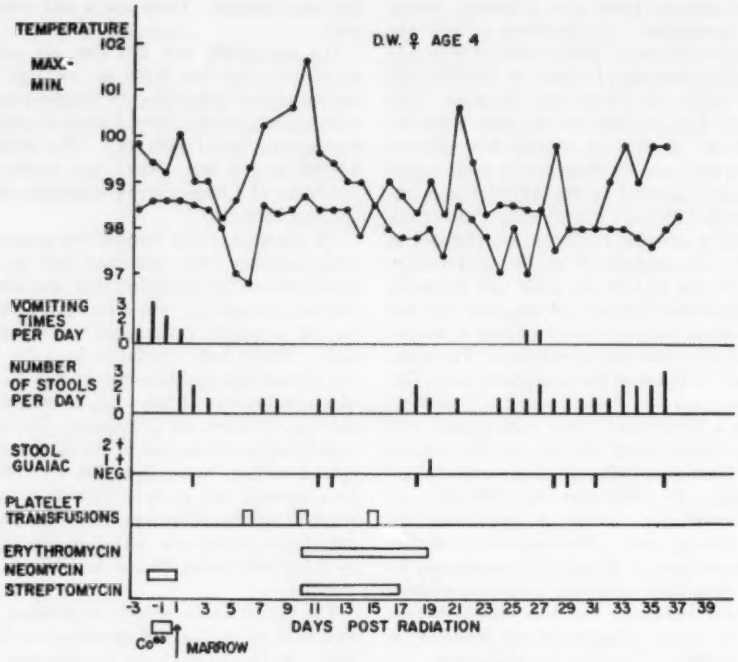

FIG. 3B. CLINICAL EVENTS IN CASE 2

iliac crests. This marrow was transferred immediately to Hanks' solution containing 0.1 mg. of Connaught heparin[3] per ml. It was passed through stainless steel screens and administered immediately to the patient. The total number of nucleated marrow cells after correction for nucleated cells in the peripheral blood was 3.9×10^8. There was no reaction to the administration of the marrow. Examination of smears of this marrow showed a normal differential.

The hematologic events are shown in Figure 3A. Returning marrow function was evident by the thirteenth day. A bone marrow aspiration was performed on the thirtieth day after irradiation. The cellularity was normal. Normal megakaryocytes were present. The myeloid-erythroid ratio was 2 to 1. The maturation sequence of both myeloid and erythroid cells was normal, and no abnormal cells could be found. Plasma cells could not be identified. The impression was normal marrow except for the absence of plasma cells.

The clinical events are shown in Figure 3B. The patient's appetite improved. She was up and about and active around her room. A throat culture on the seventh day after irradiation showed a staphylococcus aureus sensitive to erythromycin. On the ninth day she had fever. On the tenth day she was started on streptomycin and erythromycin. Fever disappeared and these drugs were discontinued on the eighteenth postirradiation day. On the twenty-first postirradiation day she developed mild nausea and fever. Her mother, who was in constant attendance, had at this time a moderate gastroenteritis. Patient and mother promptly recovered without therapy. The patient continued to be normal and was discharged from the hospital on January 22, 1959, the thirty-seventh postirradiation day.

She was then well until 12 weeks after irradiation when she developed bone pain. A marrow aspiration disclosed recurrent leukemia. Prednisone® was administered with a subsequent partial remission, 18 weeks after irradiation at the time of writing.

DISCUSSION

The twins described in this report were assumed to be identical on the basis of appearance and identity of blood types. Their histocompatabilities were not verified by skin transplantation. Transplantation of skin from the sick twin to the well twin was not advisable because of the possibility of transplanting leukemia if the twins were identical. A skin transplant from the well twin to the sick twin would have been difficult to interpret because of the variable effect of leukemia and steroid therapy on survival of skin transplants (10). Finally, there was little time to be lost since both leukemic patients were rapidly becoming worse.

In identical twins there is no immunologic marker to distinguish donor and recipient. Hence, a successful marrow transplant must be identified

by indirect proof—in this case, comparison with the course of events expected in the absence of a successful transplant. In treating leukemics with whole body irradiation and homologous marrow transplants, we have observed one patient whose own marrow resumed function after 325 r., but only after a lapse of 21 days (3). We have irradiated several patients with doses above 400 r. Subsequent homologous marrow transplants were unsuccessful, and regeneration of the patient's own marrow did not occur within 30 days. A group of normal individuals accidentally exposed to large sublethal doses of radiation have shown returning marrow function only after five weeks (11). A patient given 200 r. followed after a week by 250 r. in preparation for a kidney transplant also required four weeks for a return of marrow function (12). From these observations one may conclude that the return of marrow function in less than two weeks in the patients described here, who received 850 and 1,140 r., is indicative of a successful transplant of isologous marrow.

In mice, isologous marrow transplants are achieved without difficulty (1). We have observed almost no sickness and a return of marrow function in 7 to 10 days in dogs receiving autologous marrow and up to 1,500 r. of whole body irradiation (13). The benign clinical course of the patients described here indicates that isologous, and, by analogy, autologous transplants are as easy to achieve in man as in other animal species. A dose of two or three billion cells appears adequate.

The eventual return of leukemia in both our patients was disappointing but not unexpected. Burchenal, Hemphill, Holmberg and Wiegand (14) observed that transplanted leukemia in mice could not be sterilized by 1,000 r. given at a rate of 86 r. per minute. Several investigators have failed to cure leukemia in the mouse with doses of radiation on the order of 900 r. followed by transplantation of isologous marrow (15, 16). Atkinson, Mahoney, Schwartz and Hesch (17) have observed a remission of two months in a leukemic patient after 200 r. followed by isologous marrow. McGovern, Russell, Atkins and Webster (18) have observed a remission of three months in a leukemic patient who received 600 r. followed by an infusion of autologous marrow that had been stored during a previous remission. It appears then that neither sublethal nor supralethal (up to 1,140 r.) whole body irradiation will cure leukemia, barring the possibility of an unusually sensitive leukemia, but will produce a significant remission in patients who have relapsed despite conventional chemotherapy. The best result that we have had is a remission of six months after 325 r. without a marrow transplant (3).

Evidently something more than radiation is needed to eradicate leukemia. Two possible approaches are suggested. First, one may transplant homologous marrow after lethal irradiation and depend on the homologous marrow to provide an immunologic environment unsuitable for survival of the leukemia (19). This approach has apparently eradicated leukemia in some mice (15, 16). However, these mice subsequently have a high incidence of death from delayed foreign marrow disease due to a reaction of the graft against the host. Whether delayed foreign marrow disease will be either serious or useful in man, and whether it can be controlled by the clinical supportive measures available are questions currently being studied.

A method of circumventing foreign marrow reaction has been suggested (20) using fetal hematopoietic tissue of sufficient immaturity to be nonreactive against the host. So far grafts of fetal marrow have not been successful in man (21). Even if successful, it is possible that they may be tolerant of the leukemia as well as of the host and thus be of no benefit in eradicating leukemic cells.

The second approach to the problem of eradicating leukemia lies in the observation that with chemotherapy and X-ray the cure rate of transplantable leukemia in the mouse is an inverse function of the number of cells present—the smaller the number of leukemic cells, the greater the possibility of cure (22, 23, 24). This suggests that the patient in remission, with a relatively small mass of leukemic cells, is an advantageous subject for radiation. It further suggests that chemotherapeutic agents may be more effective if administered during an immediate postradiation period when the number of leukemic cells is relatively small.

SUMMARY

Leukemia has been studied in two sets of identical twins. One leukemic twin was irradiated

with 850 r. and the other with 1,140 r. from Co^{60} sources. Each was then given bone marrow from the respective normal twin. Successful transplantation of this isologous marrow was determined by the return of marrow function, evident after less than two weeks, and by a benign clinical course following radiation. Leukemia recurred after remissions of seven weeks in one case and 12 weeks in the other.

From these two patients our conclusions are as follows: *1*) Transplants of isologous marrow are readily achieved in man. *2*) One thousand r. of whole body radiation does not produce troublesome acute radiation sickness in man when given at a rate of 20 to 40 r. per hour. *3*) Whole body irradiation at the 1,000 r. level produces a remission but not a cure of leukemia when followed by isologous marrow.

ACNOWLEDGMENT

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MECHANISMS OF HYPERLIPEMIA IN EXPERIMENTAL NEPHROSIS *

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Several hypotheses have been advanced to explain the hyperlipemia characteristic of the nephrotic syndrome. The work of Rosenman, Friedman and Byers (1) indicated that the liver fails to remove lipids from the circulation at a normal rate. It was suggested that a loss of lipoprotein lipase into the urine, together with a deficiency of albumin which is necessary for transport of unesterified fatty acids, leads to impaired lipid clearing, and accumulation of lipid in the serum (2). Heymann and colleagues (3), however, measured the rate of disappearance of labeled sodium laurate from the circulation and observed no difference between normal and nephrotic animals. Marsh and Drabkin (4) presented evidence that in severe experimental nephrotic syndrome there is a redistribution of lipid within the body and an associated mobilization lipemia, while in milder degrees of disease hyperlipemia is the result of increased hepatic lipogenesis (5).

We have attempted to determine the source of the excess lipid in the serum by labeling body fats with C^{14} before the injection of nephrotoxic serum and following the changes in the radioactivity of body fat compartments during the development of hyperlipemia. In addition, the rates of removal from serum of chylomicron lipid and of albumin-bound fatty acid were measured in established nephrosis.

METHODS

Production of nephrosis. Nephrosis was induced in rats by injection of rabbit antirat kidney serum prepared by the method of Heymann and Lund (6). The same lot of serum was used for all animals in each experiment. The dose was 0.4 ml. per Gm. of kidney (6), and was given in two equal injections 20 hours apart (animals sacrificed before 20 hours received only one injection of serum).

Isolation and measurement of total fatty acids and cholesterol. Liver and carcass were digested by refluxing with ethanolic KOH (1 ml. 60 per cent KOH + 2 ml. ethanol per Gm. of tissue). To obtain the cholesterol, an aliquot of the digest was extracted three times with ether (1 volume). The pooled extracts were evaporated to dryness, the residue dissolved in acetone and cholesterol precipitated from solution with a fivefold excess of 0.5 per cent digitonin in 50 per cent ethanol. The precipitate was dissolved in glacial acetic acid and aliquots were taken for counting and for chemical determination (7). A standard curve was prepared with cholesterol digitonide.

The aqueous phase remaining after extraction of cholesterol was acidified and fatty acids were extracted with petroleum ether. The extracts were evaporated to dryness and made up to a known volume in ethanol. Fatty acids were determined gravimetrically in an aliquot of the ethanolic solution.

Serum. Two ml. aliquots were saponified with ethanolic KOH. Cholesterol was extracted with petroleum ether and purified by precipitation of the digitonide. The precipitate was dissolved in glacial acetic acid and aliquots were taken for chemical determination and counting as previously described.

The aqueous phase was acidified and fatty acids were extracted with ether. The ether was evaporated to dryness and the residue was dissolved in ethanol; fatty acids were determined on an aliquot of the ethanolic solution by oxidation with dichromate (8). Results were expressed in terms of palmitic acid. There was good agreement between the results of the chemical determination and direct weighing of fatty acids.

Serum unesterified fatty acids (UFA) were measured by the method of Gordon (9).

C^{14} chyle. This was prepared by the method of French and Morris (10), using tripalmitin-1-C^{14}. Animals received intravenously 1 ml. of chyle containing 36 mg. of lipid and 5×10^5 cpm.

Radioassay. Aliquots of the fatty acid and cholesterol fractions were plated onto aluminum discs with lens paper and counted in a windowless proportional flow gas counter. All samples were corrected for self-absorption from a curve constructed with C^{14}-labeled fatty acids which were isolated from the liver of a rat previously injected with sodium acetate-1-C^{14}.

Electrophoresis. Paper electrophoresis was carried out on Whatman paper No. 3 in veronal buffer of pH 8.6 and ionic strength 0.075, at room temperature. The dried strips were stained with bromphenol blue, scanned in an automatic integrating densitometer, then cut into 2 mm.

* Supported by a grant from the National Research Council, Ottawa, Canada.

segments which were placed in the flow counter for measurement of radioactivity. Starch block electrophoresis was performed by the method of Sehon, Harter and Rose (11). After separation of proteins the block was cut into segments of 0.5 cm. width, and the proteins eluted. The eluate was analyzed for protein (12) and for radioactivity.

Animals were adult male Sprague-Dawley rats weighing 225 to 250 Gm. at the onset of the experiments. When fed by gavage the diet used was 57 per cent carbohydrate, 24 per cent protein and 15 per cent fat; this was given in two daily feedings of 12 ml. each at 9 a.m. and 5 p.m. There was free access to water at all times. The animals were housed five to a cage, in a room maintained at a constant temperature of 78 ± 1° F. and relative humidity of 40 to 50 per cent.

RESULTS

A. *Incorporation of acetate-1-C^{14} into lipids*

In a preliminary experiment rats were injected intraperitoneally with carrier-free sodium acetate-1-C^{14} (6.6×10^6 cpm dissolved in 5 ml. water) and sacrificed at intervals thereafter. It was found that incorporation of radioactivity into fatty acids and cholesterol of serum, liver and carcass was maximal between two and seven hours after the injection. In subsequent experiments zero time was therefore taken as four hours after administration of labeled acetate.

B. *Relation of lipid specific activities in liver, serum and depots during development of hyperlipemia*

Animals were adapted to tube-feeding over a period of 14 days. Two hours after a regular feeding, they were injected intraperitoneally with 1 ml. of carrier-free sodium acetate-1-C^{14} in water (approximately 35×10^6 cpm). Four hours later (zero time), a group of five animals was sacrificed. The remainder was randomly divided into two groups, one of which was given antikidney serum to induce nephrosis, the other, saline. Feeding was continued on the previously established schedule. Subgroups of five or six animals from each group were sacrificed at intervals up to 72 hours after zero time. The sera, livers and carcasses, respectively, of each subgroup were combined and fatty acids and cholesterol extracted from each pool. The final subgroups of animals were sacrificed at 96 hours; in this case the extractions were carried out on individual nephrotic livers and sera, but on pooled carcasses.

Following injection of antikidney serum, fatty acids and cholesterol increased markedly in serum (the differences between the regressions of lipid on time were significant at the 1 per cent level for both fatty acids and cholesterol), and moder-

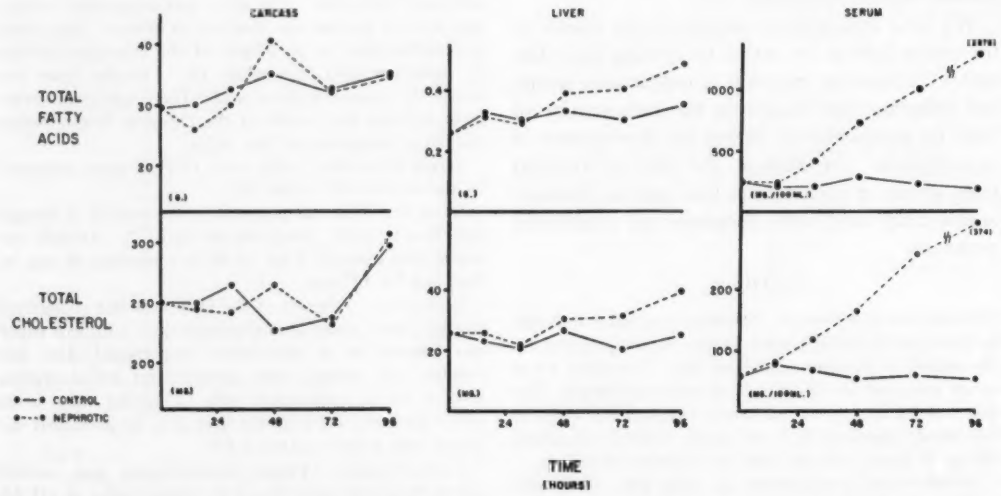

FIG. 1. CHANGES IN FATTY ACIDS AND CHOLESTEROL FOLLOWING INJECTION OF ANTIKIDNEY SERUM

Each point represents mean value of 5 to 6 animals. Carcass and liver values are for total lipid content; serum values are for concentration. (Solid line, control animals; broken line, nephrotic animals.)

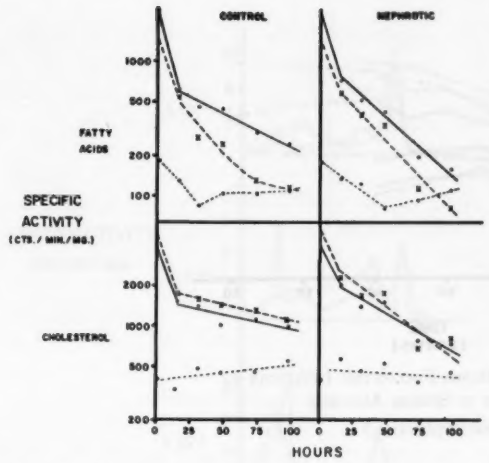

FIG. 2. CHANGES IN SPECIFIC ACTIVITY OF LIPIDS DURING DEVELOPMENT OF NEPHROTIC HYPERLIPEMIA

Animals were given acetate-1-C^{14} to permit labeling of endogenous lipids, and four hours later (Time 0 in figure) injected with antikidney serum (·——· liver; —— serum; ·–·–· carcass).

ately in liver ($p < .01$ for fatty acids and $< .02$ for cholesterol); carcass lipids did not differ significantly from those of control animals (Figure 1). The time courses of specific activities (Figure 2) differed in normal and nephrotic animals.

(*a*) *Fatty acids.* In normal rats, serum specific activity fell rapidly to the level of carcass specific activity; in plotting log specific activity against time, the line of best fit was found to be curvilinear, represented by the equation $Y = 2.9398 - 0.0169X + 0.0000807X^2$, with significant deviation from linearity ($p < .05$). The liver specific activity fell rapidly during the first 15 hours, then more slowly in an exponential manner with a rate constant $(K)^1$ of $-.0046$. In nephrotic rats after 15 hours the liver specific activity fell more rapidly than normal ($K = -.0086$) in a curve almost parallel to that of serum ($K = -.0110$). The serum specific activity in all nephrotic animals at 96 hours was lower than that of the pooled carcass fatty acids. The carcass specific activity in both groups showed an initial fall, then a plateau.

(*b*) *Cholesterol.* In both normals and nephrotics, following an initial rapid decline, the specific activities of serum and liver fell in a parallel manner, but more rapidly in the latter group; the rate constants for serum and liver, respectively, were $-.0024$ and $-.0020$ in the normals and $-.0064$ and $-.0053$ in nephrotics. Changes in carcass specific activity were not marked in

[1] K = change in log specific activity per hour.

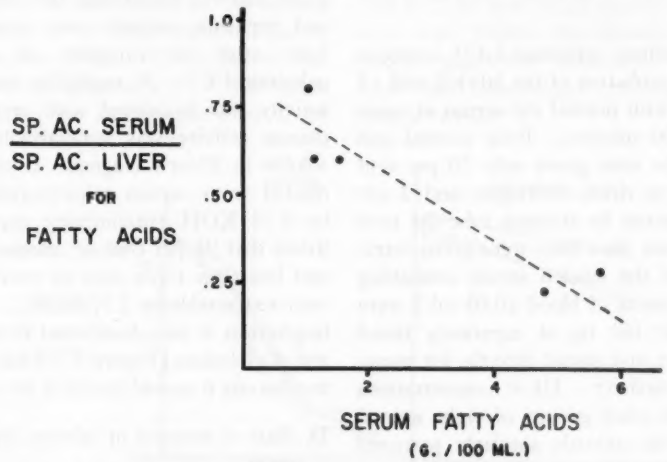

FIG. 3. RELATION BETWEEN DEGREE OF HYPERLIPEMIA AND RATIO:

$$\frac{\text{SPECIFIC ACTIVITY SERUM FATTY ACIDS}}{\text{SPECIFIC ACTIVITY LIVER FATTY ACIDS}}$$

Values are from nephrotic rats 96 hours after injections of antikidney serum.

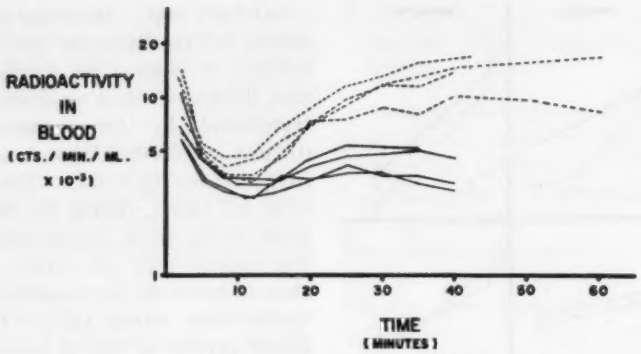

FIG. 4. RADIOACTIVITY IN WHOLE BLOOD FOLLOWING INJECTION OF PALMITIC ACID-1-C^{14} BOUND TO SERUM ALBUMIN
(Solid line, control animals; broken line, nephrotics.)

either case, and the rate constants did not differ significantly from zero.

In nephrotic rats sacrificed at 96 hours there appeared to be an inverse relationship between concentration and specific activity of serum fatty acids. To overcome the influence on specific activity of variations in initial incorporation of C^{14} into lipids, the ratio: specific activity serum fatty acids/specific activity liver fatty acids was plotted against serum lipid concentration and an inverse relationship was again indicated (Figure 3).

C. *Rate of disappearance of unesterified fatty acid from serum*

An albumin-sodium palmitate-1-C^{14} complex was obtained by incubation of the labeled acid (1 mc. per mMole) with normal rat serum at room temperature for 30 minutes. Four normal and four nephrotic rats were given only 50 per cent glucose in water to drink overnight, and 3 ml. of 50 per cent glucose by stomach tube the next morning. One hour later they were given intravenously 1 ml. of the labeled serum containing 1 μc. of C^{14}. Aliquots of blood (0.05 ml.) were obtained from the tail tip at accurately timed intervals thereafter and plated directly for measurement of radioactivity. UFA concentrations were measured in other groups of eight normal and eight nephrotic animals similarly prepared with 50 per cent glucose.

In both normal and nephrotic rats the concentration of radioactivity fell rapidly in the first five minutes, reached a minimum value at 10 to 15 minutes and then began to rise (Figure 4). There was no gross difference in the initial rates of fall between the two groups. However, the level of activity reached in the secondary rise was considerably higher in the nephrotic than in the control animals.

Serum unesterified fatty acid concentrations (mean $\pm$ S.D.) were 0.11 ± 0.02 mEq. per L. for normal rats and 0.15 ± 0.07 mEq. per L. for nephrotics. The difference was not significant at the 5 per cent level.

In order to determine the nature of the radioactive material re-entering the circulation, normal and nephrotic animals were exsanguinated one hour after an injection of albumin-sodium palmitate-1-C^{14}. A negligible amount of radioactivity was associated with erythrocytes. The plasma activity was nondialyzable and entirely soluble in Bloor's reagent. It could not be extracted from carbon tetrachloride-ether (18:1) by 1 N KOH (preliminary experiments established that 95 per cent of unesterified fatty acids and less than 1 per cent of esterified fatty acids were extractable by 1 N KOH). On paper electrophoresis it was distributed in the regions of α and β globulins (Figure 5), while on starch electrophoresis it moved ahead of the albumin.

D. *Rate of removal of labeled chylomicrons from serum*

Normal and nephrotic rats were prepared with 50 per cent glucose feedings as above, and then injected with 1 ml. of labeled chyle containing 46

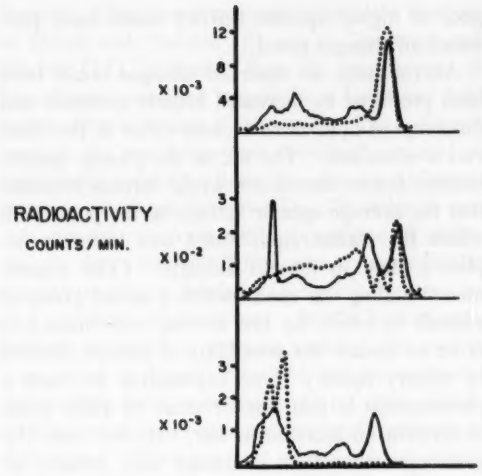

FIG. 5. ELECTROPHORETIC DISTRIBUTION OF RADIOACTIVITY

Top: palmitic acid-1-C^{14} bound to serum albumin, as injected into normal and nephrotic rats. Middle: plasma from nephrotic rat 60 minutes after receiving albumin-palmitate-1-C^{14} complex. Bottom: chyle containing palmitic acid-1-C^{14} as injected into normal and nephrotic rats. In all cases the solid line represents protein concentration, the dotted line, radioactivity.

mg. lipid. Blood samples of 0.05 ml. each were taken at 5 to 10 minute intervals and plated directly for radioactivity measurement. In normal rats the time curve of C^{14} concentration had at least two components (Figure 6), similar to that found by French and Morris (10). The mean half-life (by inspection) during the interval 15 to 40 minutes was eight minutes, compared to a value of 11.5 minutes found by these workers after injection of somewhat more lipid into smaller animals. In the present experiment, sampling was not continued long enough to get a measure of the half-life for the later part of the curve. In nephrotic rats the time curve was quite different, since there appeared to be only one component with a half-life varying from 28 to 160 minutes. The chylomicron count on blood from both normal and nephrotic animals prepared with 50 per cent glucose feedings as described was lower than five.

DISCUSSION

The first experiment described above differs in two respects from similar work previously reported: body lipids were labeled prior to development of nephrosis, and dilution of labeled fats by exogenous lipids was controlled by tube feeding. Total fatty acids are heterogenous, being derived from neutral fat, phospholipid and cholesterol esters; cholesterol may be free or esterified. The results, as in earlier work, therefore reflect the averages of the changes within these subclasses. In addition, the specific activities determined experimentally were means for each of the three anatomical pools, since each such pool may consist of more than one "functional" pool, each with its own initial specific activity and turnover rate. Within the framework of these facts certain qualitative conclusions may be drawn from the relationships among the curves of specific activity.

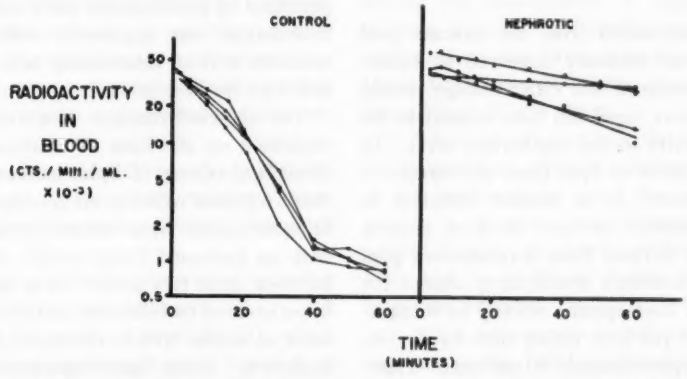

FIG. 6. RADIOACTIVITY IN WHOLE BLOOD FOLLOWING INJECTION OF C^{14}-LABELED CHYLE, CONTAINING 36 MG. OF TOTAL LIPID

With regard to fatty acids, the rapid fall in liver specific activity during the first 15 hours suggests that this organ contained at least two separate pools, one having a very high turnover rate, the other(s) a lower rate (Figure 2). A similar situation may also have obtained in the carcass but the data are not as definite. It can reasonably be assumed that an insignificant amount of lipid synthesis or degradation occurred in the serum, and that the decrease in serum specific activity was entirely due to replacement by lipid from lower specific activity sources. The shape of the specific activity curve in serum may be explained by several hypotheses, one of which is that serum lipid was derived in part from the rapidly metabolizing pool in the liver and in part from a carcass pool whose specific activity was close to that of the whole carcass and whose turnover rate was low.

In the nephrotics there occurred: (*1*) increases in the lipid content of serum and liver, (*2*) an increase in the rate of fall of liver specific activity, (*3*) a fall in serum specific activity below that of the whole carcass. These changes may have been due to (*a*) retention of lipid in the serum, (*b*) increased mobilization of lipid to the plasma, and subsequent movement of part of this "excess" lipid to the liver, (*c*) increased synthesis and discharge of lipid by the liver, or (*d*) a combination of these.

Retention of lipid in the serum would not of course account for the increase in lipid content of the liver; if hyperlipemia were due to retention alone, there must also have occurred an increase in synthesis or a decrease in degradation of lipid in the liver.

Increased mobilization from the carcass pool with which serum normally appeared to equilibrate, in the absence of any other change, would have caused a more rapid fall than normal in serum specific activity to the equilibrium level. In addition, mobilization of lipid from the carcass to liver, via the serum, in an amount sufficient to produce the observed increase in liver content (even if entirely derived from a constituent pool of zero specific activity) would have altered the rate constant for liver specific activity by a maximum of 20 to 25 per cent rather than by the observed value of approximately 90 per cent. However, mobilization together with an increase in the rate of interchange between serum and a hepatic pool of higher specific activity could have produced all changes noted.

Alternatively all observed changes could have been produced by increased hepatic synthesis and discharge of lipid alone or with either of the other two mechanisms. The fall in the plasma specific activity below that of the whole carcass indicates that the average specific activity of the pools with which the plasma equilibrated was lower in nephrotic than in normal animals. (The experimental finding was confirmed in a second group of animals in which the last feeding was omitted in order to obviate the possibility of isotope dilution by dietary lipids.) One explanation for such a phenomenon is that one or more of these pools underwent an increase in size, turnover rate (by pathways other than exchange with serum) or both. In the case of the liver this could explain the increases in lipid content and in the rate of fall of specific activity of the whole organ, as well as the inverse relationship between serum lipid concentration and the ratio: $\frac{\text{serum specific activity}}{\text{liver specific activity}}$.

As anticipated from the fact that the liver is the major site of cholesterol synthesis and that there is normally a constant interchange of cholesterol between liver and serum, there was in normal rats a close similarity between specific activities of cholesterol in these two pools. In principle, the considerations applied to the hypotheses of retention and hepatic synthesis of fatty acids are equally pertinent in the case of cholesterol. However, one need not here postulate an increase in the rate of interchange between serum and liver as an accompaniment of mobilization, since the normal rate of interchange was apparently sufficiently high to maintain a close relationship between the specific activities in these pools.

The observed changes then may be adequately explained on the basis of increased hepatic synthesis and release of lipid, increased hepatic synthesis together with retention of lipid in the serum, or mobilization from carcass to serum and liver, with an increased (fatty acids) rate of exchange between these two pools. It is apparent that the hypotheses of mobilization and of decreased movement of serum lipid to the liver (1) are mutually exclusive. Since later experiments did indicate the occurrence of "retention," we believe that mobilization did not play a role in producing hyper-

lipemia. This view is compatible with the findings of Marsh and Drabkin (5) and of Heymann and Hackel (13), indicating that the development of hyperlipemia resulted largely from increased lipid synthesis and release by the liver. They did not exclude the possibility that decreased removal of lipids from serum by liver was also an etiological factor in the hyperlipemia (1). In order to test this concept, the rate of disappearance of radioactivity from the circulation was measured after administration of labeled UFA-albumin complex and of labeled chylomicrons. In the former case, because of its initial extreme rapidity, the rate of removal of radioactivity could not be measured accurately; there was, however, no gross difference between the normal and nephrotic animals in this regard. That this was not an artifact due to differences in isotope dilution is indicated by the similarity in UFA concentrations.

There was a distinct decrease, however, in the rate of removal of chylomicron lipid from the serum of nephrotic rats. Isotope dilution was not a factor in this result since the dietary preparation of the animals reduced endogenous chylomicron levels to a minimum value. The results of the chyle experiment are compatible with those of early work in human nephrotic subjects (14, 15) and with those of Friedman, Rosenman and Byers on the metabolism of ingested cholesterol (16). They are in contrast to those of Heymann and associates (3), who injected intravenously labeled trilaurin together with synthetic emulsifiers and observed no difference between normal and nephrotic rats in the rate at which radioactivity left the blood stream. However, this was not a physiological form of serum lipid and its behavior may not reflect that of endogenously synthesized lipoproteins. Furthermore, the fate of intravenously administered emulsions is dependent on the particular emulsifying agent used (17). This does not argue against the recent observation (18) that urine contains an agent which reduces hyperlipemia; this agent may be lipoprotein lipase itself or a cofactor (1, 19).

It has been shown that triglycerides, including those of chylomicrons, may be converted to UFA by the action of lipoprotein lipase (20–23), which is released into the plasma following ingestion of lipid (24). It has been suggested (25) that a major factor in producing hyperlipemia in nephrosis is an inhibition of lipoprotein lipase activity secondary to the decrease in the amount of serum albumin available to act as an acceptor of UFA released by the enzyme. The present results are compatible with this view. However, since most chylomicron lipid is removed without prior conversion to UFA (26), it is likely that the defect is one of tissue rather than plasma lipoprotein lipase. These possibilities are presently being investigated.

An interesting incidental finding after administration of UFA-albumin complex was the reappearance of radioactivity in increased amount in the circulation of nephrotic rats. Positive identification has not been made of the form of this radioactivity, but in agreement with recent work in man (27), the evidence available suggests that it is associated with esterified fatty acids. This is further presumptive evidence of increased hepatic synthesis and turnover of lipid in nephrosis.

The present experiments indicate that there are at least two defects in lipid metabolism to account for the development of hyperlipemia in experimental nephrosis: (*a*) increased entry of lipid into the serum, and (*b*) decreased ability to remove chylomicron lipid from the circulation.

SUMMARY AND CONCLUSIONS

1. Normal rats were given injections of acetate-1-C^{14} to permit endogenous labeling of fatty acids and cholesterol. Some of the animals were then injected with anti-kidney serum to produce nephrosis, and the distribution of radioactivity among body lipid compartments measured at intervals during the development of hyperlipemia. The changes in content and specific activity can best be explained on the basis of increased hepatic synthesis and discharge of lipid together with retention of lipid in the serum.

2. After intravenous injection of palmitic acid-1-C^{14} bound to albumin, nephrotic rats disposed of the radioactivity from the serum at a rate similar to normal. The radioactivity later reappeared in the circulation, probably as esterified fatty acid, to a greater degree in nephrotic than in normal animals.

3. After intravenous injection of chyle containing tripalmitin-1-C^{14} radioactivity disappeared from the circulation of nephrotic rats at a much lower rate than normal.

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STUDIES ON THE RENAL CONCENTRATING MECHANISM. IV. OSMOTIC DIURESIS*

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Studies of osmotic diuresis in the hydropenic state have demonstrated a decrease in urine solute concentration as urine flow increases (1, 2) but the physiologic mechanism of this decrease is not fully understood. Although concentrating ability appears to be limited by a maximal osmotic urine/plasma (U/P) ratio, small increases in solute excretion have been shown to lead to a decrease in osmotic U/P ratio even when urine flow remains less than 2 ml. per minute (3). At high flows, when there is a marked decrease in osmotic U/P ratio, there is evidence that the concentrating mechanism may be limited by a constant maximal rate of net water reabsorption ($T^c_{mH_2O}$) (4). In the present study the response to osmotic diuresis has been examined over a wide range of urine flow in multiple experiments in the same subject. As urine flow increased up to 20 ml. per minute per 1.73 M.² there was a consistent curvilinear decrease in U/P ratio and a curvilinear increase in net water reabsorption ($T^c_{H_2O}$). These curves could be described by simple reciprocal equations from which maximal values for U/P ratio and for $T^c_{H_2O}$ could be predicted.

It has been suggested that the response of the concentrating mechanism to urea diuresis differs from that obtained with other osmotic diuretics (5–8). By the present method of analysis the response to urea and mannitol loading did not differ at intermediate rates of urine flow. At very high flows, on the other hand, the curvilinear relationship between urine flow and concentrating response was disrupted so that $T^c_{H_2O}$ decreased with isotonic mannitol loading and increased with urea loading, while hypertonic mannitol loading gave intermediate values.

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METHODS

The subjects were healthy young men who had demonstrated an ability to empty their bladder consistently at low rates of urine flow. The subjects were deprived of water for 18 to 20 hours prior to each experiment and given a dry supper and no breakfast. Solute loading was begun in the morning after two or three preliminary urine collection periods, and was generally maintained at a constant rate for two or more hours in order to obtain data during a steady state. Subsequent analysis indicated that data obtained with steady, increasing and decreasing rates of urine flow were sufficiently similar to be pooled. Aberrant values were obtained in the initial period after loading when urine flow increased more than twofold and these periods were discarded. The solutions of urea or mannitol used for intravenous loading also contained sodium chloride in concentrations of 0.1 to 0.7 per cent to minimize sodium losses. Hence all infusions were actually hypertonic to plasma. Sufficient vasopressin was added to provide 200 milliunits per hour, except at the highest rates of urine flow (more than 20 ml. per minute) when the dose of vasopressin was increased to 600 milliunits per hour. The following types of experiments were performed:

1. Isotonic mannitol loading. Five per cent mannitol was infused at rates of 3 to 20 ml. per minute with a constant infusion pump or at rates of 40 to 80 ml. per minute by gravity using a large bore needle.

2. Hypertonic mannitol loading. Fifteen per cent mannitol was infused at 10 or 20 ml. per minute by a constant infusion pump. Initial loading with hypertonic mannitol led to severe postural headache and therefore this infusion was given only after a diuresis had first been established by infusing isotonic mannitol at 20 ml. per minute for two hours.

3. Urea loading. Thirty per cent urea was infused at 1 to 2 ml. per minute. A few attempts at more rapid administration led to severe postural headache and nausea.

4. Urea plus mannitol loading. In order to compare the effects of urea and mannitol at high urine flow, 6 per cent urea in 5 per cent mannitol was infused at 20 ml. per minute.

Chemical methods have been described previously (3). All data were corrected to 1.73 M.² of body surface area. Concentrating ability is evaluated both in terms of the osmotic U/P ratio and in terms of net water reabsorption, $T^c_{H_2O}$, calculated as follows:

$$T^c_{H_2O} = \frac{U_{osm}V}{P_{osm}} - V. \qquad 1)$$

$U_{osm}V/P_{osm}$ represents the volume of fluid that would

TABLE I

*Relationship between urine flow (V), osmotic urine/plasma ratio (U/P) and net water reabsorption ($T^c_{H_2O}$) during osmotic diuresis**

Subject, age, surface area	No. of periods	Range of values V	Range of values U/P	Range of values $T^c_{H_2O}$	a	b	r	Predicted $T^cmax_{H_2O}$	Actual $T^cmax_{H_2O}$	Predicted U/P max	Actual U/P max
		ml./min./1.73 M.²						*ml./min./1.73 M.²*			
Pooled data†											
M. G., 25, 1.90 M.²	63	1.9–19.3	2.8–1.4	3.3–9.0	.106	.391	.985	9.43	9.32	3.56	4.18
P. M., 25, 1.98 M.²	47	1.9–19.7	3.0–1.3	3.4–7.7	.126	.282	.980	7.94	7.88	4.55	3.96
A. M., 23, 1.59 M.²	52	2.0–19.4	2.9–1.3	3.5–7.5	.138	.250	.978	7.25	7.24	5.00	4.43
Individual expts.‡											
J. F., 34, 2.04 M.²	15	4.5–17.4	1.9–1.3	3.8–6.6	.137	.554	.980	7.30	6.42	2.81	3.54
W. A., 28, 1.55 M.²	21	1.8–17.1	2.5–1.3	2.9–4.5	.204	.290	.987	4.90	5.26	4.49	3.82
R. F., 25, 2.06 M.²	12	3.6–14.3	2.3–1.5	4.7–7.1	.114	.505	.974	8.77		2.98	4.53
J. P., 26, 1.90 M.²	12	3.7– 9.6	2.2–1.6	4.4–6.1	.124	.409	.990	8.07		3.45	4.37

* The regression constants (*a* and *b*) and the correlation coefficient (r) are given for Equation *2*. Predicted $T^cmax_{H_2O}$ and U/P max are calculated from Equations *2* and *3* and compared with actual maximum values obtained in the same subjects at urine flows greater than 20 and less than 0.5 ml. per minute per 1.73 M.², respectively.

† Data from five or more loading experiments including isotonic mannitol, hypertonic mannitol, urea, and urea plus mannitol.

‡ Isotonic mannitol loading in J. F. and W. A., hypertonic mannitol loading in R. F. and J. P.

contain the urinary solutes in a solution isosmotic with the plasma, the osmolal clearance (C_{osm}). If net water reabsorption is the final operation in urine formation, then C_{osm} can be considered to represent the load of isotonic fluid delivered from the distal tubule to the concentrating site and $T^c_{H_2O}$ the reabsorptive activity at that site. Since there is recent evidence that solute transport occurs even in the terminal portions of the collecting system (9), it may not be exact to equate C_{osm} with load but $T^c_{H_2O}$ is still a measure of *net* water economy. The statistical methods used are described by Snedecor (10).

RESULTS

As urine flow increased from 2 to 20 ml. per minute per 1.73 M.² during osmotic diuresis, there was a curvilinear decrease in osmotic U/P ratio, and an increase in $T^c_{H_2O}$ (Figure 1). Pooled data for multiple experiments in the same subject showed relatively little scatter, so that it appeared feasible to fit these curves to a single regression equation. The most linear representation of the data was consistently obtained when the reciprocal $\frac{1}{U/P - 1}$ was plotted against V (Figure 1). The regression equation for this relationship:

$$\frac{1}{U/P - 1} = aV + b \qquad 2)$$

was calculated by the method of least squares.[1] From this equation it can be predicted that as V approaches zero, osmotic U/P ratio should approach a limiting maximum value (U/P max) which will depend on the value of the constant *b* $\left(U/P\ max = \frac{1}{b} + 1\right)$. Dividing Equation *2* by V and substituting Equation *1* we obtain:

$$\frac{1}{T^c_{H_2O}} = a + \frac{b}{V}. \qquad 3)$$

This equation predicts that as V increases, $T^c_{H_2O}$ will approach a maximum value ($T^cmax_{H_2O}$) which will depend on the value of the constant *a* $\left(T^cmax_{H_2O} = \frac{1}{a}\right)$. These equations provide a description of the relationship between flow and concentrating ability over a wide range of urine flow. Using Equation *2* various groups of data could be compared in terms of their regression constants, or from U/P max and $T^cmax_{H_2O}$ since the latter are directly derived from these constants. Table I gives these values for the subjects of Figure 1, and for individual experiments over a wide range of urine flow in four additional subjects. The correlation coefficients are uniformly high and the values of $T^cmax_{H_2O}$ show good agreement with the highest values of $T^c_{H_2O}$ actually attained in the same subjects at urine flows of 20 ml. per minute or more. The maximal U/P ratios obtained in these subjects were quite uniform while the predicted values showed greater variation.

In a previous study (3) differences in the response to urea and mannitol loading were observed at very low urine flows but these differences were associated with variations in the U/P

[1] This equation was derived empirically. For theoretical basis, see discussion.

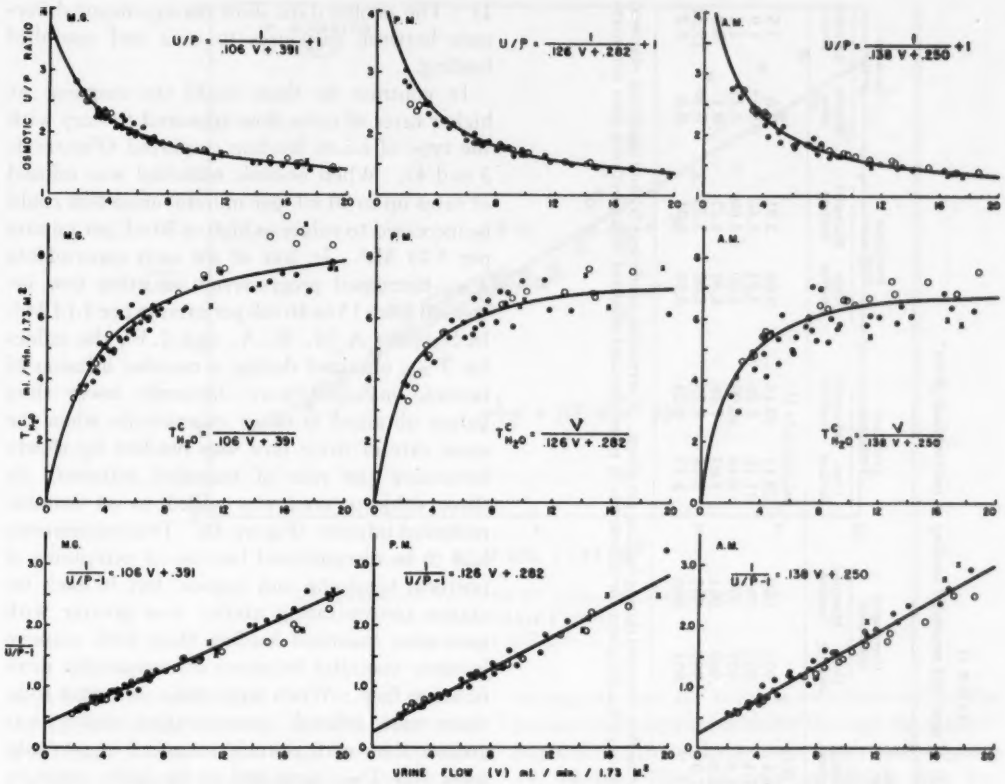

FIG. 1. CONCENTRATING RESPONSE AT URINE FLOWS (V) UP TO 20 ML. PER MINUTE PER 1.73 M.² IN THREE SUBJECTS DURING LOADING WITH ISOTONIC MANNITOL, UREA AND HYPERTONIC MANNITOL

● Isotonic mannitol; ○ urea; × hypertonic mannitol. Equation *2* relating $\frac{1}{U/P-1}$ to V, calculated by the method of least squares, is represented in the lowest graph, and rearranged to give the relationship between U/P or $T^c_{H_2O}$ and V in the upper graphs.

ratio attained prior to loading. In the present study five pairs of experiments were obtained in which U/P ratios were similar prior to loading (Table II, Figure 2), although the range of urine flow during loading was greater with mannitol diuresis. Sufficient data were obtained at intermediate urine flows in each experiment to calculate the regression equation *2*. By analysis of co-variance (10) the values of $\frac{1}{U/P-1}$ were analyzed for the effects of changing urine flow (regression), for random deviations from regression and for differences in the effect of urea and mannitol loading (adjusted means). While differences in regression (represented in Table II by differences in $T^c max_{H_2O}$) were probably significant on the paired experiments on Subjects P. M. and A. M., these were in opposite directions in the two subjects. The experiment in which the highest urine flow was achieved with urea loading (T. T. No. 2) showed a greater concentrating response to urea than to mannitol loading which was statistically highly significant. In terms of osmotic U/P ratio, the mean difference was only 0.16. Differences as large as this at intermediate urine flows have been observed in the response to repeated isotonic mannitol infusions in the same subject (see A. M., Figure

TABLE II

Paired experiments on the effects of urea and mannitol loading at intermediate flows *

Subjects	Number of periods		Highest U/P ratio before loading		Highest urine flow during loading		Correlation coefficient		Predicted $T^c max_{H_2O}$		Predicted U/P max		Variance ratios	
	Urea loading	Mannitol loading	Urea loading	Mannitol loading	Urea loading	Mannitol loading	Urea loading	Mannitol loading	Urea loading	Mannitol loading	Urea loading	Mannitol loading	Regression	Adjusted mean
					ml./min./1.73 M.²				*ml./min./1.73 M.²*					
M. G.	7	8	3.67	3.90	4.2	6.4	.972	.989	10.11	10.10	3.60	3.91	0.6	3.5
P. M.	7	8	4.02	4.14	4.6	7.4	.915	.993	11.76	8.26	3.24	4.46	5.4†	1.9
A. M.	7	7	3.80	4.07	4.3	7.4	.977	.999	8.48	10.99	4.60	3.60	8.4†	1.2
T. T. No. 1	10	12	4.16	3.89	3.6	3.6	.969	.961	7.63	7.46	4.31	4.27	0.1	0.8
T. T. No. 2	14	10	3.36	3.58	7.4	7.9	.989	.992	8.48	8.09	4.00	3.33	0.4	79.5‡
All data	45	45					.974	.939	8.73	8.54	3.95	3.91	0.3	1.2

* The highest U/P ratio attained before loading, and the highest urine flow attained during loading are given. Correlation coefficient (r) and predicted maxima, $T^c max_{H_2O}$ and U/P max are obtained from Equations *2* and *3*. Variance ratios are given for differences in regression and adjusted mean between urea and mannitol loading experiments.

† Significant ($p < 0.05$).

‡ Highly significant ($p < 0.001$).

1). The pooled data show no significant difference between responses to urea and mannitol loading.

In contrast to these data, the response at higher rates of urine flow appeared to vary with the type of solute loading employed (Figures 1, 3 and 4). When isotonic mannitol was infused at rates up to 80 ml. per minute, urine flow could be increased to values as high as 50 ml. per minute per 1.73 M.². In five of six such experiments $T^c_{H_2O}$ decreased progressively as urine flow increased from 15 to 40 ml. per minute per 1.73 M.². In Subjects A. M., W. A., and J. F., the values for $T^c_{H_2O}$ obtained during a massive infusion of isotonic mannitol were distinctly lower than values obtained in other experiments when the same rate of urine flow was reached by slowly increasing the rate of mannitol infusion. In three subjects urea was added to an isotonic mannitol infusion (Figure 3). Two experiments had to be discontinued because of symptoms of postural headache and nausea, but in each instance concentrating ability was greater with urea plus mannitol loading than with massive isotonic mannitol infusions at comparable rates of urine flow. When hypertonic mannitol solutions were infused, concentrating ability was greater than with isotonic mannitol in two subjects and $T^c_{H_2O}$ appeared to be fairly constant over a wide range of urine flow.

Data on plasma and urine composition for intermediate and high flow experiments are summarized in Table III. At peak flow the sum of urinary urea, mannitol, and sodium with its anion, accounted for 96 to 99 per cent of total solute. The urine urea concentration ranged from 500 mMoles per L. during urea loading at intermediate flows to 12 mMoles per L. during mannitol loading at high flows.

DISCUSSION

The present analysis of the response of the renal concentrating mechanism to osmotic diuresis has yielded an equation relating osmotic U/P ratio and urine flow, Equation *2*, which can be rewritten as follows:

$$\frac{1}{(T^c_{H_2O})} = \frac{1}{(T^c max_{H_2O})} + \frac{1}{(U/P\ max - 1)} \frac{1}{V} \cdot \quad 4)$$

This equation closely resembles the Line-

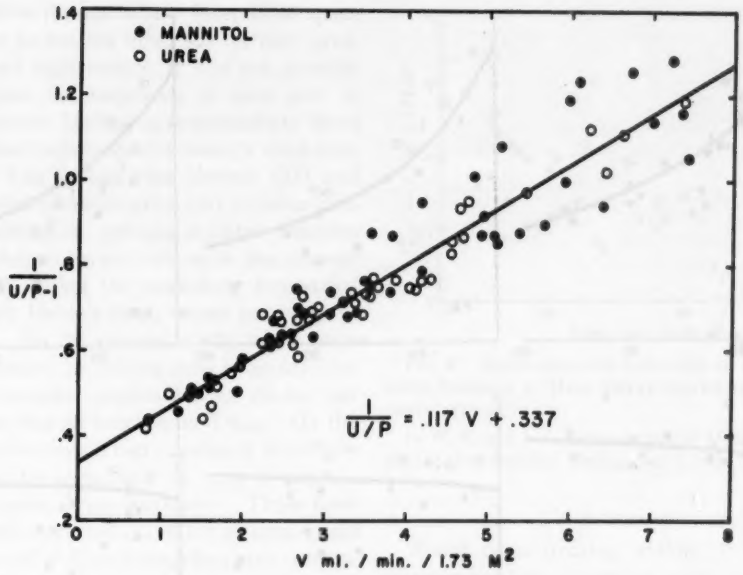

FIG. 2. CONCENTRATING RESPONSE TO UREA AND MANNITOL LOADS AT INTERMEDIATE FLOWS
Symbols as in Figure 1.

weaver-Burk modification (11) of the classical Michaelis-Menten equation for the description of enzyme kinetics. Thus $T^c_{H_2O}$ would represent a reaction velocity and V would represent substrate concentration, with $T^cmax_{H_2O}$ and U/P max representing velocity and concentration maxima, respectively.[2] This analogy need not imply that urine is concentrated by enzymatic active reabsorption of water. There is evidence to suggest that the urine is concentrated by the passive reabsorption of water through the collecting ducts into a hypertonic peritubular space (5, 14–16). The manner in which this hypertonicity is achieved is not certain but it presumably requires active sodium transport (17). It is therefore possible that the resemblance of the equation describing the concentrating mechanism to a Michaelis-Menten equation is a reflection of the limitations imposed on the concentrating mechanism by an enzyme system involved in sodium transport. On the other hand, the equations relating water reabsorption and urine flow are also analogous to the Langmuir adsorption isotherm (18, 19). This equation describes the adsorption of gases but may have application to osmotic transport of solvents in which the initial step may be represented as an adsorption of the solvent on a semipermeable membrane (20). Passive osmotic transfer could thus be limited by the osmotic gradient at low flows and by the tubular adsorbing surface area at high flows. The equations relating concentrating response to urine flow are merely descrip-

[2] In the original analysis of this relationship (12, 13) C_{osm} was taken as a measure of "substrate" and the "Michaelis-Menten equation" was written:

$$\frac{1}{T^c_{H_2O}} = \frac{1}{a'} + \frac{b'}{C_{osm}} \qquad 5)$$

in which $T^c_{H_2O}$ and C_{osm} are as described above and a′ and b′ are constants. For statistical analysis the derived terms were transformed into directly measured terms yielding the expression:

$$V = \frac{a'}{\frac{U}{P} - 1} - \frac{a'b'}{\frac{U}{P}}. \qquad 6)$$

The correlation between V and $\frac{1}{U/P - 1}$ was not improved by including the third term $\frac{1}{U/P}$, so that the simpler expression of Equation *2* was selected for further use.

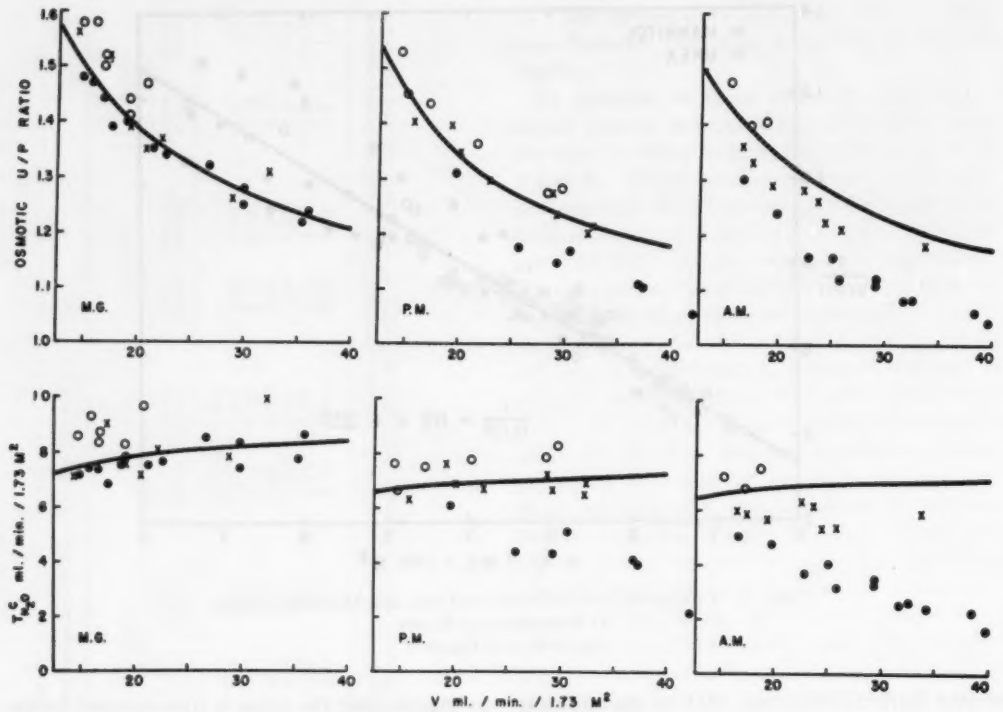

FIG. 3. CONCENTRATING RESPONSE AT HIGH URINE FLOWS
Symbols as in Figure 1. Curves continued from those of Figure 1.

tive and might be the result of still other mechanisms than the two described above.

Berliner, Levinsky, Davidson and Eden (5) have postulated that the collecting duct is permeable to urea, so that the urinary urea can be osmotically balanced by urea which has passively diffused into the medullary interstitial fluid. This circumstance should result in enhanced concentrating ability during urea diuresis since the sodium chloride which is presumably actively

TABLE III

*Plasma and urine composition at peak urine flow during various types of loading in subjects M. C., P. M. and A. M.**

Type of loading	P_{osm}	P_{urea}	P_{man}	P_{Na}	Per cent of urine osmolality		
					Urea	Mannitol	Na × 2
	mOsm/Kg.	*mMoles/L.*	*mMoles/L.*	*mEq./L.*			
Intermediate flow							
Urea	312	25	0	134	69	0	28
Mannitol	287	5	23	126	15	56	26
High flow							
Urea plus mannitol	329	36	31	111	36	35	28
Isotonic mannitol	299	5	70	96	4	66	26
Hypertonic mannitol	311	4	57	117	3	66	28

* Mean values are given for plasma osmolality (P_{osm}), urea (P_{urea}), mannitol (P_{man}) and sodium (P_{Na}), and for the per cent of urine osmolality contributed by urea, mannitol and sodium with its anion (Na × 2).

concentrated in the medullary interstitial space would be free to balance other solutes than urea. In the present experiments, it was not possible to differentiate the responses to urea and to isotonic mannitol loading at intermediate flows despite marked differences in urinary urea concentration. The finding that chronic (21) and acute (22) urea administration can enhance concentrating ability in protein depleted subjects could be explained by an increase in the amount of urea which enters the medullary interstitial space through Henle's loop, either passively or actively (8). In the present study when urine flow was increased by adding urea to an infusion of isotonic mannitol maintained at 20 ml. per minute, there was an increase in $T^c_{H_2O}$. On the other hand, when much higher rates of urine flow were attained by increasing the rate of isotonic mannitol infusion, $T^c_{H_2O}$ decreased. These findings could indicate that an effect of urea could only be detected at high flows when urea concentration was markedly decreased with mannitol loading alone. In addition, the difference between isotonic mannitol and urea loading at high flows appeared to be at least in part ascribable to differences in the osmolality or the volume of the fluid administered, since hypertonic mannitol infusion did not cause the decrease in $T^c_{H_2O}$ which occurred with isotonic mannitol loading. The data of Zak, Brun and Smith (4) also show that $T^c_{H_2O}$ decreased progressively with increasing flow in five of their seven experiments with isotonic mannitol loading. A progressive decrease in $T^c_{H_2O}$ at very high flows need not represent a decrease in actual rate of water reabsorption at the concentrating site. Micropuncture studies in the rat show that during antidiuresis the fluid entering the distal tubule is hypotonic to the plasma and only gradually becomes isotonic as it passes through the distal tubule (23). At very high flows, this osmotic equilibration might be incomplete so that hypotonic fluid is delivered to the concentrating site. The difference between the responses to isotonic and hypertonic mannitol loading in the present experiments suggests that acute expansion of extracellular fluid volume might decrease concentrating activity; however, large infusions of isotonic sodium chloride have not impaired concentrating ability acutely at lower urine flows (24).

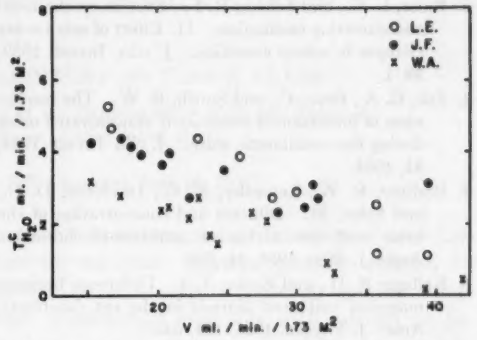

FIG. 4. CONCENTRATING RESPONSE TO ISOTONIC MANNITOL LOADING AT HIGH URINE FLOWS ON THREE ADDITIONAL SUBJECTS

In W. A. and J. F. higher values of $T^c_{H_2O}$ were obtained during slow mannitol loading (see Table I).

SUMMARY

Renal concentrating ability during osmotic diuresis has been examined over a wide range of urine flow in hydropenic normal men. As urine flow (V) increased up to 20 ml. per minute per 1.73 M.² there was a curvilinear increase in net water reabsorption ($T^c_{H_2O}$) and a curvilinear decrease in osmotic urine/plasma (U/P) ratio, which fit the equation $\frac{1}{U/P - 1} = aV + b$, where a and b are constants related to maximum net water reabsorption and maximum U/P ratio, respectively. Data obtained with urea and mannitol loading at intermediate flows fit this equation and showed no difference between the responses to the two solutes.

When urine flow was increased up to 40 ml. per minute per 1.73 M.², this relationship was disrupted. $T^c_{H_2O}$ usually decreased progressively with isotonic mannitol loading. Concentrating ability was greater with urea plus mannitol loading at these urine flows than with isotonic mannitol alone, while the response to hypertonic mannitol loading was intermediate.

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STUDIES OF THE RENAL EXCRETION OF MAGNESIUM IN MAN * †

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Magnesium is one of the four principal cations in extracellular fluid, and in intracellular fluid is exceeded only by potassium; yet comparatively little is known about the mechanisms involved in the renal excretion of magnesium. That such is the case must be due to a variety of factors which include difficulties in chemical analysis, the binding by protein of a relatively large portion of the ion in serum, and the formation of soluble complexes which may be handled by the kidney in a different manner from that of the ion. Such difficulties probably account for the relatively few studies to date of magnesium excretion.

The experiments reported here involve 70 acute studies in man performed by usual clearance techniques. Renal hemodynamics and excretion rates of magnesium and other electrolytes were observed during multiple control periods and following eight principal experimental procedures. These were intravenous infusions of sodium lactate, sodium bicarbonate, acetazolamide, probenecid, sodium para-aminohippurate (PAH) to tubular saturation, magnesium salts, calcium salts and mercurial diuretics.

The data provide a basis for examination of factors likely to affect magnesium excretion and in some instances of probable renal mechanisms involved. The major part of filtered magnesium is reabsorbed by the tubules. The existence of a quantitatively small, but perhaps important, regulatory tubular secretion of magnesium is suggested by certain indirect evidence, but is not settled by present data. In addition to changes in filtered magnesium, consideration is given to possible effects on tubular magnesium transport of such factors as acid-base balance, possible relation to known active transport systems, specific interionic relationships especially with calcium or potassium, and formation of soluble magnesium complexes.

METHODS

General. Fifty-three normal men between the ages of 18 and 32 served as experimental subjects for one or more renal clearance studies by standard techniques (4) with bladder catheterization and standard water loading. Control results reported are averages from three or more periods of at least 15 minutes each which followed an equilibration period of at least 45 minutes.

Test procedures. The test procedure in each case consisted of the intravenous administration of some electrolyte solution or pharmacological agent. The eight major types of experiments are described below. Additional procedures were carried out to elucidate specific points and will be mentioned in appropriate sections. *1) Sodium lactate,* 2.4 mEq. per Kg. body weight, as a hypertonic (0.9 M) solution was given rapidly (10 minutes) in six studies. Whenever results of lactate administration are referred to without qualification in this report it should be understood that these are the studies meant. In four other studies alterations were made in the dosage or time of administration of lactate for comparison with the main series. *2) Sodium bicarbonate* was given to three subjects in dosage equivalent to the main lactate series. *3) Maximal rates of PAH transport* by the tubules (Tm_{PAH}) were achieved by standard techniques and observations at Tm_{PAH} were made in four studies for evaluation of the effect of PAH itself. In five additional studies after first attaining Tm_{PAH} the same hypertonic lactate infusion described previously was given to determine if the response was altered by prior PAH saturation. *4) Probenecid* (Benemid®) in total dosage of 3 Gm. was infused in seven subjects. Each study included three parts: control observations, measurements of the effect of probenecid alone, and then of the hypertonic lactate response after probenecid. *5) Acetazolamide* (Diamox®) was given intravenously in dosage of 5 mg. per Kg. body weight in 10 studies. *6) Mercurial diuretics* as 2 ml. of either meralluride (Mercuhydrin®) or mersalyl (Salyrgan®) were given in 15 studies. *7) Magnesium sulfate* was infused at rates not exceeding 1 Gm. per hour in five studies. *8)*

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† Portions of this work have been reported in preliminary abstracts (1–3).

‡ Established Investigator of the American Heart Association.

Calcium was infused over a 20 minute period as calcium chloride (0.7 Gm.) in five studies and as calcium gluconate (2 Gm.) in two studies.

Chemical determinations. Inulin, PAH, creatinine and phosphate were determined by methods previously indicated (5). Magnesium was determined by a titan yellow colorimetric method based on the methods of Heagy (6) and of Orange and Rhein (7). Following mercurial diuretics, aberrant results for urinary magnesium were found and subsequently shown to be due to an interference with the titan yellow method. If the mercury was first separated by means of H_2S precipitation, the method gave satisfactory results. The gluconate ion was also found to interfere with the titan yellow method. For this reason calcium chloride experiments were carried out in place of further calcium gluconate infusions and only these results are reported. Check of the magnesium by an independent phosphate precipitation method showed that changes in magnesium excretion, as well as those of the other ions, were very similar with either calcium salt. Calcium was determined by the Tisdall method as modified by Clark and Collip (8). Sodium and potassium were determined with a Baird internal standard flame photometer, chloride by the Volhard-Harvey modified method (9). Ultrafiltrate was obtained from the serum in a few studies by means of the capsule of Lavietes (10). In some studies the urine pH was determined on a Beckman pH meter. In most the pH was estimated to the nearest 0.1 unit by use of an appropriate short-range test paper. The latter method, while not suitable where precise knowledge of pH is necessary, was found to check well with a more accurate anaerobic method, the difference seldom exceeding ± 0.2 unit (11). Plasma pH was determined with a Beckman pH meter.

Statistical methods and graphical presentation. The excretion rates for each individual were expressed as a per cent of his control rate[1] and because of the large volume of data involved it was considered desirable to present only averages. For the purpose of testing statistical significance, nonparametric methods were used. These methods are somewhat less powerful (*i.e.*, less likely to demonstrate "significance") than the better known corresponding parametric "t" test. However, since fewer assumptions are necessary, significance that is demonstrated can be considered to have a more general application. The methods are considered more reliable for small samples and it is not necessary to make the assumption that the population distribution is normal in shape. Significance of changes from control rates was tested by the Wilcoxon matched-pairs signed-ranks test (13, 14). For testing significance where paired replicates were not available (*e.g.*, comparing results of two different test procedures) the Mann–Whitney U test was used (13). Confidence limits of 94 per cent were accepted rather than the 95 per cent convention for "significance" usual for parametric tests. The reason for this is that p values less than 0.06 cannot be obtained by the nonparametric methods used with five or fewer cases and that the results of these tests have somewhat greater generality than the parametric tests.

[1] A preliminary survey of the data showed a distinct tendency with most electrolytes under the conditions studied for the size of the change in different individuals to show a positive relation to the control rate as tested roughly by the quadrant sum test of Olmstead and Tukey (12).

RESULTS

The mean urinary excretion of magnesium during control periods for all studies was 6.3 μEq. per minute. Variability "between cases" (standard deviation of mean control values for individual studies) was ± 2.6 μEq. per minute; variability "within cases" (standard error of observation calculated as the standard deviation of individual measurements about the mean value for each individual) was ± 12.2 per cent of control.[2] The corresponding values for control plasma magnesium for the entire series were: mean, 1.83 mEq. per L.; S.D. ± 0.27 mEq. per L.; and S.E. of observation ± 3.6 per cent of control.

Effects of lactate administration

The results of the "main lactate series" are presented in Tables I and II and in Figure 1. The rate of magnesium excretion rose promptly to 227 per cent of the control rate during the infusion (*i.e.*, at time 8 minutes), reaching a peak of 261 per cent about 10 minutes later. It then fell back quite rapidly, reaching the control level within one and one-half hours. The excretion of other ions also increased, but somewhat less rapidly except for calcium and sodium (which was being infused). Also, in contrast to the response of magnesium, the excretion rates of sodium, potassium, chloride and phosphate all remained elevated. In absolute terms magnesium response was small relative to that of other ions but in terms of per cent of control the changes were comparable. Plasma magnesium changed only slightly and if anything decreased somewhat, perhaps secondary to urinary loss or to hemodilution. In any event,

[2] If a given experimental procedure were to cause neither any systematic change in the excretion of an electrolyte nor any change in variability from the control period, then there would be a 95 per cent chance that any randomly chosen single measurement would fall within the tolerance interval ± 2.2 S.E. of observation from that individual's mean control rate.

TABLE I

Effects of hypertonic sodium lactate, individual experiments*

Expt.	Time†	GFR‡	Urine flow	Urine pH	Urinary electrolyte excretion						Clearance§ Mg
					Mg	Ca	Na	K	PO_4	Cl	
	min.	*ml./ min.*	*ml./ min.*		*μEq./ min.*	*μEq./ min.*	*μEq./ min.*	*μEq./ min.*	*μMole/ min.*	*μEq./ min.*	*ml./ min.*
1	−49										
	0	126	5.8	5.4	5.3	2.3	256	123	3.1	272	3.14
	+12	140	4.1		15.2	8.5	769	130	12.0	346	9.39
	+26	125	4.7	8.0	14.9	13.9	1,147	241	24.4	336	9.40
	+39	141	2.9	8.4	9.7	9.2	808	251	27.0	239	6.32
	+54	116	2.2	8.4	5.5	2.2	565	200	19.9	207	3.57
	+70	121	2.2	8.4	5.1	2.2	516	203	17.9	178	3.26
	+84	125	2.5	8.4	5.8	0.8	580	220	18.9	192	3.68
	+98	125	1.8	8.4	5.9	2.3	497	182	15.6	148	3.75
2	−52										
	0	119	9.8	6.0	6.8	0.9	241	119	26.2	284	4.04
	+17	124	11.5	7.4	18.1	20.6	1,234	183	31.7	375	11.10
	+31	114	5.7	7.8	11.9	12.9	1,065	246	34.8	366	7.55
	+48	112	2.8	8.0	8.7	6.1	648	288	30.2	250	5.33
	+64	125	2.2	8.4	9.1	5.0	556	288	28.5	204	5.61
	+80	114	1.5	8.4	7.6	3.8	421	246	21.2	134	4.51
	+94	111	1.1	8.6	6.4	2.3	328	228	15.3	108	3.50
	+110	102	1.2	8.6	6.0	2.4	301	205	13.9	88	3.59
3	−42										
	0	131	7.3	5.4	4.1		82	61	12.3	45.3	2.71
	+11	130	5.6		7.5		222	62	16.8	47.9	4.79
	+26	96	2.6	5.4	13.9		530	65	16.2	69.7	8.92
	+42	132	1.5	8.4	8.4		405	120	25.1	84.4	5.55
	+57	128	1.2	8.4	5.2		316	114	20.6	72.7	3.53
	+72	139	1.5	8.4	5.1		346	128	21.7	74.6	3.47
	+87	166	2.5	8.4	4.0		378	210	23.9	86.9	2.72
	+102	143	3.1	8.4	3.6		299	175	19.1	70.9	2.41
4	−44										
	0	123	9.3	5.4	2.6	1.5	99	61	13.1	65	1.49
	+12	130	11.7		5.3	14.9	509	72	11.4	104	2.97
	+28	106	3.6	8.2	9.3	17.2	657	122	25.6	116	5.22
	+42	127	2.3		5.8	10.3	522	157	25.2	116	3.39
	+58	141	3.0	8.4	4.8	8.7	537	180	24.2	142	2.82
	+72	132	3.0		3.7	6.3	465	155	19.9	126	2.20
	+86	136	3.6	8.4	4.1	6.2	522	151	18.3	135	2.41
	+100	118	4.3		3.0	3.9	428	154	13.4	116	1.77
5	−63										
	0	130	5.3	6.4	7.9	6.6	224	74	3.1	224	4.02
	+17	143	13.6	6.9	19.6	11.6	1,004	127	4.8	392	10.90
	+30	164	6.5	7.9	18.2	36.9	1,250	209	5.8	429	9.20
	+46	137	4.9	7.9	9.6	17.5	826	176	4.0	343	4.58
	+74	99	4.3	7.9	5.2	9.5	560	136	2.2	256	2.33
	+106	167	8.3	7.8	8.0	14.8	884	227	3.0	410	3.88
	+124	117	2.9	7.9	7.1		533	127	1.7	198	3.67
6	−47										
	0	90	8.5	5.4	17.9	20.3	226	99	18.8	168	7.45
	+13	104	2.2	5.4	24.8	32.4	417	101	14.2	179	11.30
	+26	112	5.3	8.2	31.1	53.7	1,045	247	28.2	272	16.00
	+40	99	3.2	8.4	23.1	34.4	768	240	29.4	251	11.80
	+56	96	3.9	8.4	21.0	29.9	842	211	24.7	333	11.00
	+73	104	4.4	8.4	20.0	30.2	908	211	20.8	344	10.70
	+87	101	4.0	8.4	18.2	27.2	850	200	28.5	292	9.60
	+109	96	1.9	8.4	16.9	25.7	743	181	19.8	249	8.74

* 2.4 mEq. per Kg. of body weight given intravenously in 10 minutes. Four additional studies with different dosage or time of administration of hypertonic sodium lactate are not included in the table.

† Data are expressed per individual periods which end at the time indicated, measured from the start of lactate infusion. The initial period is the average of three control periods.

‡ Glomerular filtration rate (inulin clearance).

§ UV/P, plasma magnesium uncorrected for binding to plasma proteins.

TABLE II

Summary of mean electrolyte excretion rates following various test procedures

Procedure, (No. studies) Electrolyte	Mean control	Excretion rate					
		+8 min.*	+20 min.	+36 min.	+50 min.	+65 min.	+80 min.
	μEq./min.	*Mean, % control*					
Sodium lactate (6)							
Mg	7.4	227†	261†	161†	124	112	109
Ca	6.3	745†	790†	437†	314	248	215
Na	188.0	384†	530†	391†	344†	328†	345†
K	89.7	129†	199†	226†	218†	210†	237†
PO_4	12.8¶	167	256	265	218†	197	204
Cl	176.5	137†	155†	143	142	133	138
Sodium bicarbonate (3)‡							
Mg	6.5	141	115	101	84	84	71
Ca	5.9	393	370	250	176	124	107
Na	196.9	439	460	335	341	295	181
K	74.0	241	332	284	264	250	228
PO_4	14.9¶	115	132	153	142	135	144
Cl	166.0	208	155	140	132	130	82
PAH-saturation (4)‡§							
Mg	6.8	136	161	178	187	159	166
Ca	7.0	214	322	367	390	346	323
Na	225.8	160	217	253	278	275	197
K	149.2	122	132	120	112	124	91
PO_4	16.4¶	157	202	171	156	152	151
Cl	271.2	91	86	85	84	86	92
Probenecid (7)§							
Mg	6.0	118†	108	108	121		
Ca	6.1	131	107	110	136†		
Na	149.7	167†	167†	175†	192†		
K	72.4	120	114	107	112		
PO_4	7.4¶	107	94	65†	56†		
Cl	160.3	148†	144†	148	158†		
Acetazolamide (10)							
Mg	6.9	102	87	84	92	96	98
Ca	10.9	194†	206†	211†	217†	228†	220†
Na	207.5	313†	400†	361†	345†	349†	320†
K	74.5	330†	471†	428†	387†	341†	307†
PO_4	13.0¶	157†	205†	224†	232†	204†	190†
Cl	208.9	192†	216†	212†	197†	162	144
Mercurials (15)‖							
Mg	4.8	190†	225†	263†	317†	402†	468†
Ca	6.2	226†	233†	328†	411†	536†	644†
Na	139.8	234†	323†	441†	564†	724†	880†
K	62.8	93	91	81†	77†	72†	74†
PO_4	16.4¶	132†	136†	127†	132†	125	121†
Cl	126.7	212†	281†	408†	498†	670†	873†
Calcium chloride (5)							
Mg	6.3	195	137	156†	176†	199†	190†
Ca	7.2	163†	211†	275†	348†	361†	345†
Na	123.7	146†	161†	167	168†	169†	152†
K	94.0	109	95	69	69	71	72
PO_4	12.9¶	100	93	78	80	78	63†
Cl	154.6	135	149†	153†	158†	168†	149†
Magnesium sulfate (4)‡							
Mg	13.5	169	189	327	428		
Ca	11.8	181	220	286	336		
Na	211.2	178	200	205	224		
K	162.8	76	77	72	75		
PO_4	19.8¶	160	210	209	205		
Cl	188.2	114	110	121	134		

* Measured from start of the test procedure.
† Significant change from control, Wilcoxon matched-pairs signed-ranks test.
‡ Not tested for significance because of small number of studies.
§ Effects of lactate after PAH-saturation or probenecid are not included (see Figure 4).
‖ Mersalyl (six studies) or meralluride (nine studies).
¶ Micromoles per minute.

the increased urinary excretion does not result from shift of magnesium into plasma, since any change in plasma magnesium concentration is in the other direction. No increase in proportion of ultrafiltrable magnesium was found in the two cases so studied. The increased urinary magnesium after lactate could not be attributed to changes in glomerular filtration rate, effective renal plasma flow or rate of urine flow. Changes in these factors were small and in opposite directions in individual cases, although the typical magnesium response occurred in all.

In two other studies a slightly smaller initial dose of lactate (100 mEq.) was given, followed by an additional 100 mEq. over the next hour. The rapid fall-off of magnesium excretion from the sharp peak reached soon after the initial lactate was not prevented by the continued lactate infusion, suggesting that stimuli for renal magnesium conservation may be promptly elicited and quite effective.

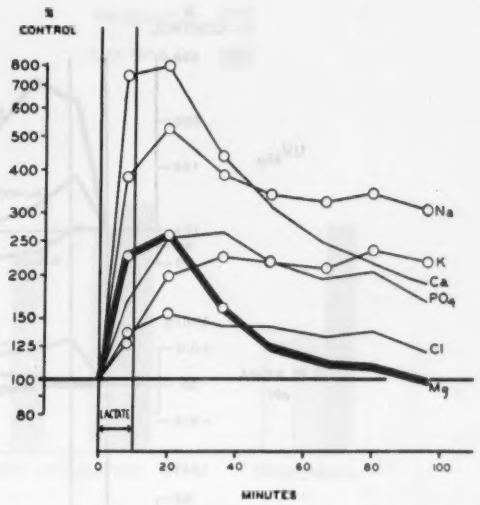

FIG. 1. RENAL EXCRETION OF ELECTROLYTES FOLLOWING INTRAVENOUS HYPERTONIC SODIUM LACTATE: MEAN CHANGES IN MAGNESIUM, SODIUM, POTASSIUM, CALCIUM, CHLORIDE AND PHOSPHATE

Each value is the mean from six experiments and is expressed as per cent of control rate. Changes from control that are statistically significant are represented by open circles.

Some comparisons with the lactate effect

Certain changes in excretion of the other major intracellular cation, potassium, are closely linked to acid-base changes. To examine the possibility of a similar relationship for magnesium the comparisons illustrated in Figure 2 were made. Three subjects were given equivalent doses of sodium bicarbonate in exactly the same manner as the usual lactate procedure. In per cent of control the average response after bicarbonate was 141 per cent at 8 minutes, 115 per cent at 20 minutes and had returned to control at 36 minutes. In none of the subjects was the peak UV_{Mg} response as large as even the smallest response in the lactate series, a significant difference between the series being demonstrated ($p = 0.02$, Mann–Whitney U test).

Figure 2 illustrates the comparative effects of lactate and bicarbonate, and also acetazolamide although other aspects of the latter will be considered separately. At the time of maximal magnesium effects, each type of procedure had produced a similar degree of alkalinity of the urine, yet the increases in magnesium excretion differed greatly in magnitude between bicarbonate and lactate and following acetazolamide there was actually a small decrease. Plasma pH similarly did not correlate well with magnesium excretion since bicarbonate had a somewhat greater effect on plasma pH than lactate in contrast to the effects on magnesium excretion. The excretion of potassium, on the other hand, appears in these data to be closely related to acid-base factors.

In two studies sodium acetate was given, the dose being reduced to one-half the usual lactate dose to avoid side reactions. A small increase in magnesium excretion (average 124 per cent of control at 8 minutes) was observed, similar in magnitude to that observed in two additional lactate studies with one-half the usual dose.

Tests of "inhibition" of the lactate response

An effect which is distinctly greater following sodium lactate than equivalent sodium bicarbonate brings to mind the relative effects of these substances on the tubular transport system handling para-aminohippurate (PAH), penicillin, phenol red and certain other substances (see Discussion). To examine a possible role of this transport system

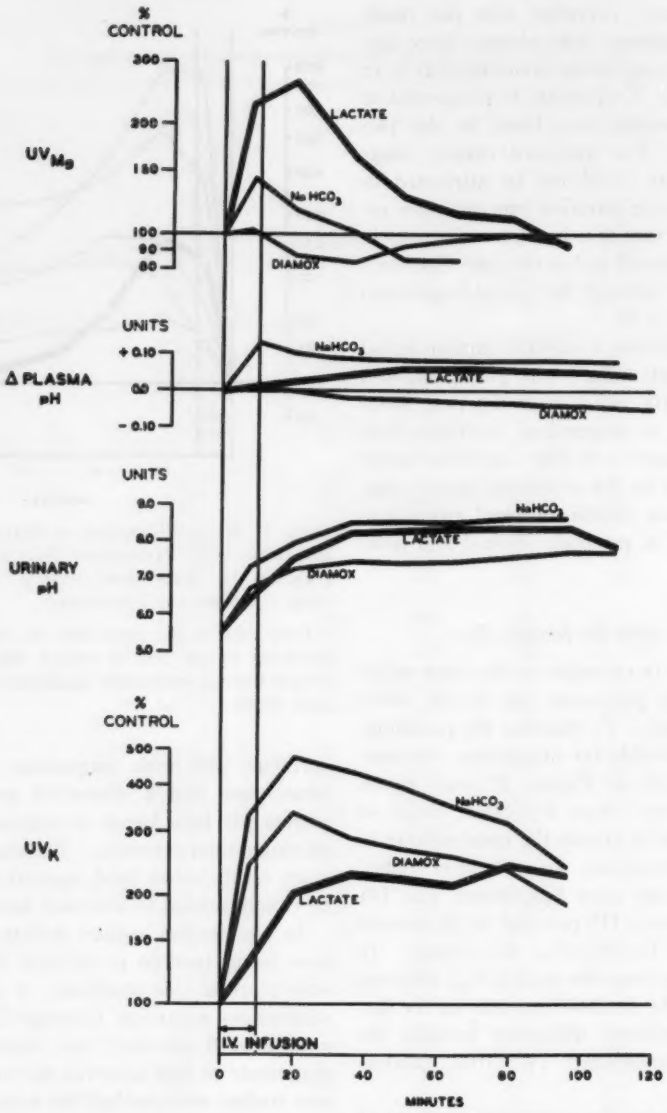

FIG. 2. COMPARATIVE EFFECTS OF LACTATE, BICARBONATE AND ACETAZOLAMIDE (DIAMOX®) ON MAGNESIUM EXCRETION, PLASMA pH, URINARY pH AND POTASSIUM EXCRETION

Mean values from six experiments with lactate, three with bicarbonate and ten with acetazolamide.

in magnesium excretion, the transport system was inhibited by probenecid or saturated by PAH administration. Results are illustrated by Figure 3. When lactate was given after either of these procedures the response in UV_{Mg} seemed to be reduced if expressed relative to the excretion after the "inhibiting" procedure. However, probenecid and PAH saturation without lactate administra-

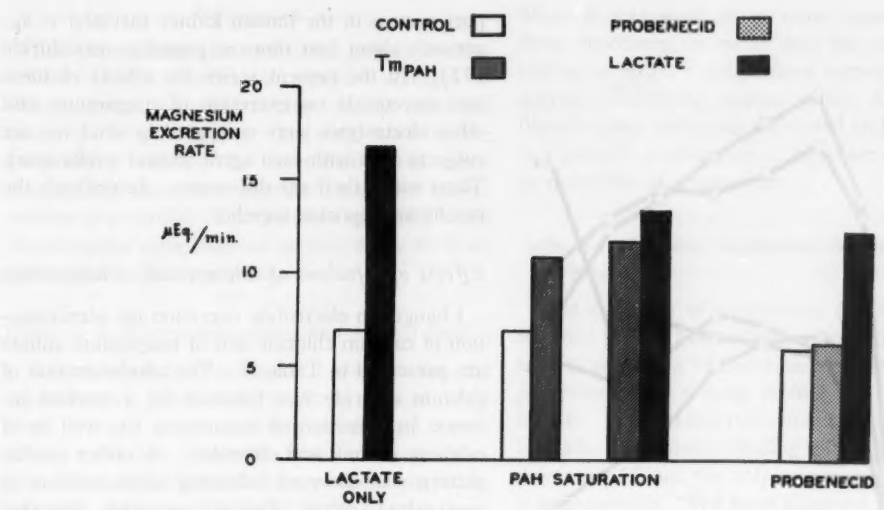

FIG. 3. EFFECTS OF PAH SATURATION AND OF PROBENECID ON MAGNESIUM EXCRETION AND ON URINARY MAGNESIUM RESPONSE TO HYPERTONIC LACTATE

Contiguous vertical bars represent mean values of consecutive parts of a single series of experiments. Lactate response is the mean at 20 minutes after start of the lactate infusion under each circumstance shown. See text for number of studies of each type.

tion appeared to cause increases in UV_{Mg} (Table II and Figure 3). The change following probenecid was extremely small, but consistent enough to reach statistical significance. When compared with the standard error of observation of 12 per cent of control it seems probable that the change after PAH saturation was also real, although the number of cases was insufficient to show "statistical significance." The average rate reached after lactate in μEq. per minute appeared somewhat smaller after both probenecid and PAH saturation than in the main lactate series. Nevertheless, expressed in these terms, in contrast to change from control, the differences between the various series were not statistically significant.

Effects of acetazolamide administration

Several features of the acetazolamide studies in addition to those mentioned above are illustrated by Figure 4 and Table II. At 20 minutes after infusion of the carbonic anhydrase inhibitor, UV_{Mg} averaged only 87 per cent of control and at 36 minutes, 83 per cent. Some decrease was observed in 9 out of 10 studies and, although small, was statistically significant for the series. This was the only one of the experimental procedures observed which caused a decrease of magnesium excretion below control levels. This decrease was closely correlated with a decrease in glomerular filtration rate, which averaged 86 and 85 per cent of control at 20 and 36 minutes, respectively.

Calcium excretion showed a distinct increase following acetazolamide. At the times of significant fall in the magnesium excretion (+ 20 and + 36 minutes) the calcium excretion rate averaged 206 and 211 per cent of control. Under other experimental conditions there was a noticeable similarity of behavior between UV_{Mg} and UV_{Ca} while comparisons of changes in excretion rates of magnesium with those for sodium, potassium, chloride and phosphate showed frequent examples of differences in time of maximum change and even sometimes in direction of change. Changes in UV_{Mg} were usually somewhat smaller both in μEq. per minute and in per cent of control than simultaneous changes in UC_{Ca}. Otherwise, the pattern of change was noticeably similar, except for the changes after acetazolamide when excretory rates of calcium as well as each of the other ions *except magnesium* showed a sharp increase.

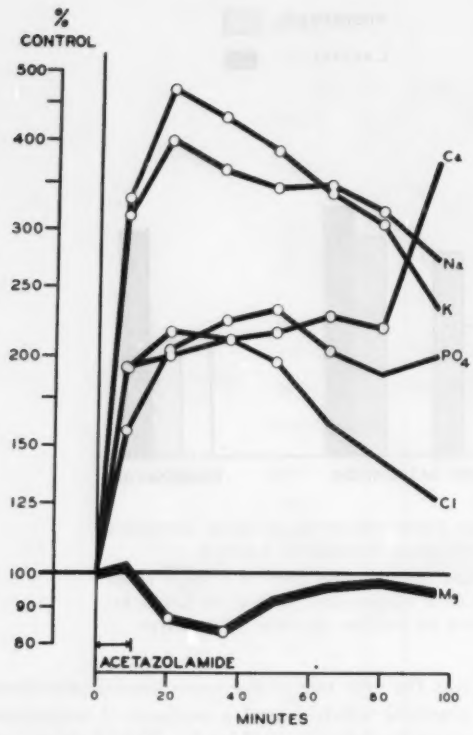

FIG. 4. RENAL EXCRETION OF ELECTROLYTES FOLLOWING INTRAVENOUS ACETAZOLAMIDE: MEAN CHANGES IN RENAL EXCRETION OF MAGNESIUM, POTASSIUM, CALCIUM, CHLORIDE AND PHOSPHATE

The data are presented as in Figure 1.

Effects of mercurial diuretics

Increased urinary excretion of magnesium was observed following injection of meralluride or mersalyl as shown in Table II. In contrast to the short peak observed following lactate, this effect was increasing throughout the period observed. The highest excretion rate observed (averaging 529 per cent of control) was at 96 minutes, which is beyond the times shown in Table II. On the average, potassium excretion decreased somewhat, a result clearly different from the distinct increase in UV_{Mg} and contributing one more situation where a definite difference was observed in the renal handling of the two major intracellular cations.

As measured by depression of the PAH transport system in the human kidney mersalyl is apparently about four times as potent as meralluride (15). In the present series the effects of these two mercurials on excretion of magnesium and other electrolytes were compared by studying six subjects once with each agent several weeks apart. There was little if any difference. Accordingly the results are reported together.

Effects of infusions of calcium and of magnesium

Changes in electrolyte excretion on administration of calcium chloride and of magnesium sulfate are presented in Table II. The administration of calcium chloride was followed by a marked increase in excretion of magnesium (as well as of calcium, sodium and chloride). A rather similar pattern was observed following administration of magnesium sulfate. Calcium excretion, like that of magnesium, sodium and chloride showed a definite increase. Changes in phosphate excretion were relatively small and variable following either magnesium or calcium administration. On the average potassium excretion decreased in both series. While this was small enough not to be statistically significant with the number of cases studied it is clearly different from the behavior of calcium and magnesium.

Ignoring the fact that part of the magnesium is bound or nonfiltrable, control excretion of magnesium was only about 3 per cent of that filtered. With the administration of magnesium it increased to only about 25 per cent of the amount filtered with the rather small infusions we were willing to give human subjects. Even with liberal allowance for protein binding it is obvious that no net tubular secretion of magnesium was demonstrated. In separate experiments on dogs we have been able to observe magnesium excretion of the same order of magnitude as magnesium filtration, making allowance for binding to plasma proteins (16). We have, however, not observed circumstances where excretion clearly exceeds simultaneous reabsorption to demonstrate a net secretion. A few observations did appear to suggest a small net secretion, but may represent experimental variation under these circumstances or the fact that the proportion of bound magnesium remains in some doubt.

DISCUSSION

The acute effects observed in the present studies are not always identical to reported changes in daily magnesium excretion after chronic administration of the same agents. The data, however, are particularly suitable for examination of renal mechanisms since they provide concurrent observations of a number of parameters at a time when physiological compensation is less likely to have obscured the primary events.

Changes in plasma magnesium and filtered load

Except when magnesium itself was infused, the experimental procedures reported caused only small changes in plasma magnesium and sometimes in different directions in different individuals. More often than not the average changes observed in these studies would cause filtered magnesium to change in the direction opposite to that in which UV_{Mg} changed.

The part of plasma magnesium bound to protein has been said to constitute anywhere from 14 to 52 per cent of the total, but most recent estimates under normal conditions (physiologic pH range, normal proteins) indicate that about 35 per cent of the total magnesium is protein bound (17–19). This ratio between ultrafiltrable magnesium and total serum magnesium probably remains relatively constant despite marked changes in total serum magnesium, a relationship previously observed for calcium (20). Changes in pH *within the ordinary physiological range* cause changes in proportion of calcium bound to protein so small as to be near or within the limits of accuracy of present experimental methods (21). From the similar way in which magnesium and calcium react with serum protein, this observation would probably also hold for magnesium. What data we have from ultrafiltration studies are consistent with these concepts. In any event it should be observed that any variations in proportion of filtrable plasma magnesium due to pH changes in our particular experiments would be expected to be in the wrong direction to account for the results observed.

In individual experiments changes in glomerular filtration rate appear to be the factor most likely to alter the rate of magnesium *filtration*. However, with most of the experimental procedures the filtration rate went up in some experiments and down in others; in either case the same type of change in urinary magnesium excretion was observed. Following acetazolamide, however, the filtration rate uniformly decreased and this change is a possible explanation of the observed decrease in excretion of magnesium.

Some observations on tubular magnesium reabsorption

The clearance of magnesium (calculated using the total plasma magnesium concentration) during control periods in 65 experiments averaged 4.0 ml. per minute with a range from 1.5 to 13.9 ml. per minute. It is evident that even if liberal allowance is made for protein binding of magnesium, the dominant (if not the only) renal tubular process is reabsorption. We have observed a number of conditions in which *decreased* tubular reabsorption is the most likely explanation of increased magnesium excretion. For example, mercurial diuretics, which are known to depress a number of tubular processes, caused a marked and sustained increase in the excretion of magnesium. None of the test procedures examined produced decreases in magnesium excretion except for acetazolamide, which also caused a fall in glomerular filtration rate. Such absence of demonstrated *increases* in reabsorbed magnesium is consistent with our previous suggestion (16), based on magnesium loading in dogs, that possibly tubular transport processes normally operate at or near saturation. The same observation, *i.e.*, that increments in filtered load of magnesium are excreted almost completely, has also been made by Chesley and Tepper on magnesium administration to pregnant and nonpregnant women (22). The conclusion that "basal" magnesium reabsorption is nearly maximal must be considered a tentative one, not only because of the indirect nature of the evidence, but because of the magnitude of possible experimental error.

Several groups are currently investigating the tubular site of magnesium transport in dogs by the stop-flow technique (23, 24) and a preliminary report indicates that ". . . magnesium is reabsorbed with greatest avidity in the distal part of the nephron at a site slightly proximal to that of maximal reabsorption of sodium and potassium" (24).

Is there a tubular secretory process for magnesium?

Hirschfelder suggested that most of the magnesium appearing in the urine is secreted by the tubules (25), an interpretation based on animal studies indicating that magnesium excretion is depressed by injury of the tubules more than by injury of the glomeruli and follows the excretion of phenolsulfonphthalein more nearly than that of xylose. He further pointed out observations of earlier workers that magnesium is excreted by the aglomerular kidneys of the toadfish. It has been indicated above that if a secretory tubular transport for magnesium exists at all in man or dog, it must be very small compared to concurrent reabsorption. Nevertheless, since it could play an important regulatory role, the question of its existence is not without importance. We have not, by magnesium loading in either men or dogs (16), encountered circumstances where "net" secretion was clearly present (i.e., where magnesium excretion clearly exceeded the rate of magnesium filtration). If changes in magnesium excretion were due principally to changes in tubular *secretion* one would anticipate that inhibition of this transport and therefore decrease of magnesium excretion below control levels would not be uncommon. Since none of our test procedures produced such decreases (except acetazolamide which also decreased filtered magnesium) regulation of tubular *reabsorption* as suggested above seems more likely. It has recently been demonstrated by radioactive magnesium injection during stop-flow analysis that nonfiltered magnesium can enter the distal nephron via the tubule (23). This may, however, represent only a back diffusion or flux in a direction opposite to the predominant movement of the ion. While a three-component system including tubular secretion is not excluded, present data may be explained by filtration and reabsorption alone.

Is magnesium excretion related to known tubular transport systems?

Lactate caused an increase in magnesium excretion which averaged approximately four times the increase observed after equivalent bicarbonate administration (Table II). Lactate also caused about four times the effect of bicarbonate on maximal tubular transport of PAH in man, both as reported earlier (26) and in a small series we have studied. Studies were therefore made of a number of other procedures known to influence the renal transport mechanism concerned with the secretion of PAH and certain other weak organic acids such as phenolsulfonphthalein, Diodrast® and penicillin. These included administration of probenecid, acetate, mercurial diuretics and PAH itself to Tm levels, and combinations of one of these procedures followed by lactate administration. Changes in magnesium excretion usually resulted, but there were enough differences between the patterns of Mg and of PAH response as mentioned above so that the nature of the relationship, if indeed there is any, is clearly not a direct one.

Another tubular secretory transport system has been recently suggested as involving synthesis and secretion of intermediates of the citric acid cycle (27). Malate secretion appears established and probably citrate and α-ketoglutarate are secreted under conditions of metabolic alkalosis (27). It is possible that magnesium might accompany such secreted organic acid, or that magnesium complexes formed after the anion reached the urine might interfere with magnesium reabsorption, but present data contribute no direct evidence to evaluation of such possibilities.

The role of soluble magnesium complexes

Magnesium, like calcium but in contrast to the univalent cations, has a marked tendency to form nonionized complexes, including chelate complexes. Only a small proportion of plasma magnesium is in the form of such soluble complexes, but magnesium excretion might be altered by any procedure that changes the concentration of complexing anions in the tubular lumen at the site of magnesium reabsorption. Present knowledge in this field is not adequate to assess the probable role of such processes. It would be necessary to know the concentrations of various potential complexing agents at the site involved, the pH at the required site, the tenacity of the bonds in the particular complexes and the relative manner in which the tubule handled magnesium ions, the complexing anions and the complex. Even if only the magnesium ion were reabsorbed the complex might not make the bound fraction unavailable for

reabsorption if immediate dissociation of complex occurred as fast as concentration of the free fraction was reduced. Similarly PAH is virtually completely cleared from plasma despite considerable protein binding.

Despite the manifest difficulty of quantifying the role of complex formation in magnesium excretion, there is some evidence suggesting that it may be important. After massive infusion of sulfate, which forms an incompletely dissociated salt with magnesium, Walser and Browder observed increased magnesium excretion in the dog from 6 to 70 per cent of filtered magnesium (28). Citrate warrants particular consideration, both because of its strong complexing property (29) and because it is responsible for the major part of the variation in urinary organic acid produced by metabolic acidosis and alkalosis (30). However, lactate infusion in dogs caused no larger change in citrate excretion than the small increase after bicarbonate (31), a result in contrast to the relative effects we observed on magnesium output. Such observations suggest that if complex formation is important in magnesium excretion it is often masked by other factors. Lactate is not likely to be of similar importance in this respect since the formation constant of the complex formed by magnesium with lactate is comparatively small (29).

Effect of acid-base changes

Although many procedures that affect acid-base balance also change magnesium excretion, our data demonstrate a clear separation between magnesium excretion and pH of either the urine or the plasma. The speculation that the pH within the tubular cells is more important might provide some improvement, but still not a good correlation with magnesium excretion. For example, comparative effects of lactate, bicarbonate and acetazolamide on UV_{Mg} (Figure 2) do not seem explicable by acid-base factors alone.

Are there specific interionic relationships, especially to potassium or calcium?

Our data do not indicate any consistent relations between excretion of magnesium and sodium, chloride, phosphate, or total ionic strength of urine.

Potassium excretion in man usually decreases following parenteral injection of magnesium salts (32, 33) (Table II). The hypothesis has been suggested that magnesium, a divalent ion, may enter into a univalent ion exchange mechanism, resulting in a decreased excretion of potassium (32). The failure of potassium chloride ingestion to produce a consistent effect on magnesium excretion does not lend support to such a suggestion (34). In contrast to the observations in man and to our own observations in dogs following infusion of magnesium sulfate, it has been reported that infusion of magnesium chloride in the dog markedly increased the excretion of potassium (24). In the present studies examination of the effects of various tests procedures (Table II) indicates that those procedures that produced the greatest changes in potassium excretion were associated with only modest changes in magnesium excretion and those procedures that produced the largest effect on magnesium excretion had comparatively little effect on potassium output, often causing a slight decrease.

Calcium and magnesium excretion showed a noticeable similarity of behavior with most of the test procedures (Table II). In Figure 1, for example, a similarity in shape of the curves for these two ions is evident. It is not clear whether this indicates some specific relation between magnesium and calcium or whether it results simply from the fact that a number of factors are likely to affect these chemically similar divalent ions in the same way. Magnesium infusion produced increased calcium excretion and calcium infusion produced increased magnesium excretion. Following acetazolamide, however, excretion of calcium increased markedly while magnesium excretion decreased somewhat. Apparently renal mechanisms regulating magnesium excretion are, at least partly, separate from those of other ions including calcium.

Effects of other factors

No consistent relationship was observed between excretion of magnesium and renal plasma flow (PAH clearance) or rate of urine flow. While changes in anion excretion may influence magnesium together with other cations in a nonspecific manner to maintain electroneutrality, such

effects are probably not predominant, as illustrated by the marked differences in response to lactate and to bicarbonate.

SUMMARY

By acute clearance techniques and various experimental procedures, in 70 studies in normal humans, factors influencing the renal excretion of magnesium were examined. Procedures included intravenous infusions of sodium lactate, sodium acetate, sodium bicarbonate, acetazolamide, probenecid, sodium para-aminohippurate (to tubular saturation), magnesium salts, calcium salts and mercurial diuretics alone and in certain combinations.

On infusion of hypertonic sodium lactate, excretion of magnesium increased to 261 per cent of control (average, six studies) within 20 minutes, then fell back rapidly, reaching the control rate within 90 minutes. Except for acetazolamide, each of the other procedures also produced an increase in magnesium excretion, although that following probenecid was quite small. The decrease following acetazolamide was coincidental with a fall in glomerular filtration rate and the increases in excretion after magnesium administration showed no definite differences from the probable increase in filtered magnesium.

Changes in glomerular filtration rate, urine flow, renal plasma flow, plasma magnesium and acid-base factors did not correlate well with magnesium excretion in most of the conditions tested. Many factors which affected the tubular transport system for para-aminohippurate also caused changes in magnesium excretion but there were enough differences to indicate that the relationship is not simple and direct.

It is suggested: *1*) that the major portion of filtered magnesium is reabsorbed by the tubules by processes operating normally at or near saturation; *2*) that such tubular reabsorption is the main regulatory process although tubular secretion cannot be excluded; and *3*) that renal mechanisms regulating magnesium excretion are, at least partly, separate from those of other ions including calcium and potassium.

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THE REMOVAL OF LIPOPROTEIN LIPASE FROM THE BLOOD BY THE NORMAL AND DISEASED LIVER * †

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Lipoprotein lipase is an enzyme found in many organs and tissues. The highest concentration is in heart and adipose tissue (2, 3). Its unique physiological substrate consists of the triglyceride component of lipoproteins which it hydrolyzes to fatty acids and glycerol (4). Thus, it differs from pancreatic lipase which will act upon triglycerides not bound in a lipoprotein complex. Hahn first noted that this enzyme appeared in the blood in a highly active form following parenteral heparin administration. It had the property of reducing the turbidity of lipemic plasma (5) and became universally known as "clearing factor." Heparin or a heparin-like substance is now thought to be a part of the lipoprotein lipase molecule (6, 7).

Although the precise role of lipoprotein lipase in the metabolism of triglycerides has not yet been defined, it has excited great interest. It may play a role in the hydrolysis of chylomicron triglyceride formed after the ingestion of dietary fats. It may be involved in the pathogenesis of atherosclerosis. It may be important in the outflow of the unesterified fatty acids which provide a significant and readily mobilizable source of energy during the fasting state (8, 9).

Jeffries (10) and later Spitzer and Spitzer (11) showed that the isolated, perfused rat liver destroyed lipoprotein lipase. Jeffries also found that hepatic venous blood of the heparinized rat had less clearing activity than did aortic blood. Morris and French, however, were unable to demonstrate loss of clearing factor by perfusing the rat liver (12). Hepatectomized and cirrhotic rats had higher plasma lipemia clearing activity than control rats (13). Baker, Levine, Turner and Dubin found that patients with hepatic cirrhosis had higher post-heparin blood levels of clearing factor than normal subjects (14). It was suggested that this increased activity might result from failure of the liver to inactivate the enzyme.

The present investigation was undertaken to define the role of the liver in the removal of lipoprotein lipase from the blood in dogs and humans. Lipoprotein lipase levels were determined in various vascular beds. In these species the normal liver removed lipoprotein lipase from the blood; liver disease prevented the usual removal.

METHODS

Animal experiments. Dogs in the fasting state, weighing from 17 to 25 Kg., were anesthetized with ether or sodium pentobarbital. Ten normal dogs were studied. Hepatic vein catheterization was performed via the external jugular vein. The catheter was placed in a branch of the hepatic vein draining the left lobe of the liver. A polyethylene catheter was introduced into a surgically exposed femoral vein for peripheral venous sampling. At the beginning of the experiment a preheparin sample was drawn from the femoral vein. Ten mg. of heparin [1] was then given intravenously. The hepatic vein catheter was kept patent by a slow saline-heparin drip. Five mg. of additional heparin was given slowly by this route during the experiment. Simultaneous femoral and hepatic venous samples were drawn at 10, 60 and 90 minutes post-heparin. Blood in 9 ml. amounts was added to 1 ml. of 1.85 per cent potassium oxalate solution in tubes chilled in an ice bath. Specimens were centrifuged at 4,000 rpm and 4° C. for 10 minutes. Subsequently, the plasma specimens were kept at 4° C. for a period not longer than two hours before enzymatic activity was determined. The enzyme remains stable in plasma for at least 24 hours at 4° C.

Hepatic necrosis was induced in three dogs by carbon tetrachloride or chloroform poisoning (15, 16). The same studies were performed as in the normal dogs. In Dogs 1 and 2 studies were performed before and after liver poisoning. All three dogs developed severe icterus,

* Presented at the annual meeting of the American Society for the Study of Arteriosclerosis in San Francisco, Calif. October 24, 1958 (1).

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‡ This work was performed during the tenure of an American Heart Association Research Fellowship.

[1] Sodium heparin, Abbott Laboratories.

anorexia and vomiting. The experiments were performed on the third day of illness. Hepatic necrosis was confirmed by gross and microscopic examination in two dogs which died later in hepatic coma.

We have assumed in these studies that the lipoprotein lipase level in peripheral venous blood is similar to the level in blood entering the liver (*i.e.*, in portal vein and hepatic artery blood). In four normal dogs this hypothesis was checked by obtaining post-heparin blood samples simultaneously from the portal vein, the femoral vein, the hepatic vein and the femoral artery. The portal vein was exposed after laparotomy. A Cournand needle was inserted into the femoral artery of these dogs for arterial blood sampling.

Human experiments. Ten human subjects were studied in the fasting state. All had been receiving the general hospital diet with regard to fat content and were eating well. Five, to be termed normals, had no evidence of liver disease. Four had mild hypertension and one had valvular heart disease. None was in cardiac failure. Five other subjects had long-standing Laennec's cirrhosis documented by physical examination, very abnormal liver function tests, and liver biopsy. Ascites, present in three patients, did not influence the results of this study. The cirrhotic patients were in good nutritional state. All had hepatic vein wedge pressures elevated to at least 20 mm. of mercury. Hepatic vein catheterization was performed by introducing a catheter into an antecubital vein and under fluoroscopic control passing it through the right atrium and inferior vena cava into a branch of the hepatic vein draining the right lobe of the liver. A Cournand needle was inserted into the other antecubital vein and in some experiments into the femoral artery. At the beginning of the experiment a preheparin sample was drawn from the antecubital vein. After the catheter was put into position in the free hepatic vein, 10 mg. of heparin was given intravenously. Simultaneous antecubital and hepatic venous samples were drawn at 10, 60, and occasionally 90 minutes post-heparin. Femoral arterial samples were also obtained in several subjects. Blood was handled as in the dog experiments.

Lipoprotein lipase activity. Plasma lipoprotein lipase was quantified in two ways: (*a*) by the reduction in optical density or "clearing" of a plasma-coconut oil emulsion and (*b*) by the measurement of glycerol produced by lipolytic action. These techniques were modified from descriptions by Grossman (17), Korn (2) and Baker (18). Coconut oil provides a suitable substrate for lipoprotein lipase despite the inability of the enzyme to act upon purified triglycerides. As Korn has demonstrated (4), the incubation of coconut oil with plasma lipoproteins results in the formation of a complex analogous to chylomicrons with respect to lipoprotein lipase action.

Plasma was warmed at 37° C. for three minutes. Then 2.5 ml. was mixed with 0.3 ml. of a coconut oil emulsion.[2] The concentration of the emulsion was adjusted so that the mixture of plasma and coconut oil gave an initial optical density reading of approximately 0.60 unit. The optical density and glycerol content of the plasma-coconut oil emulsion were determined in duplicate at zero time and after 120 minutes of incubation at 37° C. The 120 minute incubation period was selected as a suitable time for the measurement of both optical density change and glycerol production in the same incubation tube. We had found glycerol production still linear at 120 minutes. The change in optical density was linear for the lower levels of lipoprotein lipase usually found in 60 and 90 minute post-heparin specimens. The 120 minute period was long enough so that the low levels of lipoprotein lipase present in some plasma specimens could be determined. Bovine albumin was not added to the incubation system because we did not wish to mask a possible effect of the altered plasma proteins of liver disease upon lipoprotein lipase activity. Since oxalated plasma was employed, the formation of turbid calcium soaps was not a problem. In no instance did the incubation tube develop more turbidity after maximal clearing had occurred. Anfinsen's data had indicated a similar lack of returbidification after 180 minutes of incubation (19). Korn had used a 120 minute incubation period for many of his experiments employing lipoprotein lipase from rat heart (2).

The optical density was read in 10 mm. cuvettes at a wave length of 650 mμ in a Coleman Junior Spectrophotometer. For glycerol determinations 0.5 ml. aliquots of the plasma-coconut oil emulsion were added to chilled tubes containing 0.1 ml. of 0.1 N sulfuric acid. The plasma proteins were precipitated by 2 ml. of 20 per cent trichloroacetic acid. Glycerol was determined on 0.25 ml. of the filtrate by the method of Lambert and Neish (20) and expressed in μMole per ml. The results were analyzed statistically by the methods of Fisher (21).

[2] Ediol, Schendley Laboratories; kindly provided by Dr. B. M. Lamman.

RESULTS

Normal dogs

Ten healthy mongrel dogs had much higher levels of lipoprotein lipase in peripheral vein blood than in hepatic vein blood 60 and 90 minutes post-heparin. The data for each dog at 10, 60 and 90 minutes post-heparin are tabulated in Table I. No significant differences occurred at the 10 minute period for the group, but Dog 9 had considerable 10 minute peripheral vein-hepatic vein differences. These were 0.230 optical density unit and 0.54 μMole of glycerol.

At 60 minutes the optical density change for all normal dogs averaged 0.304 unit in the peripheral vein samples and 0.213 unit in the hepatic vein samples. The mean difference was 0.091 ($p < 0.001$). Glycerol production in these ani-

TABLE I

Lipoprotein lipase in normal dogs and in dogs with hepatic necrosis

Subject No.	10 min. post-heparin				60 min. post-heparin				90 min. post-heparin			
	Change in optical density		Glycerol production		Change in optical density		Glycerol production		Change in optical density		Glycerol production	
	Peripheral vein	Hepatic vein	Peripheral vein	Hepatic vein	Peripheral vein	Hepatic vein	Peripheral vein	Hepatic vein	Peripheral vein	Hepatic vein	Peripheral vein	Hepatic vein
	units		*μMole/ml.*		*units*		*μMole/ml.*		*units*		*μMole/ml.*	
Normal dogs												
1	0.479	0.480	0.67	0.68	0.272	0.172	0.38	0.22				
2	0.300	0.290	0.56	0.51	0.237	0.170	0.41	0.31	0.230	0.152	0.42	0.18
3	0.317	0.297	0.57	0.54	0.215	0.152	0.34	0.29	0.207	0.110	0.34	0.19
4	0.387	0.362	0.90	0.86	0.372	0.172	0.63	0.38	0.170	0.120	0.24	0.13
6	0.410	0.400	0.59	0.62	0.270	0.167	0.28	0.14	0.132	0.070	0.08	0.03
7	0.402	0.412	0.86	0.88	0.390	0.370	0.64	0.66	0.304	0.205	0.46	0.29
8	0.425	0.420	0.78	0.78	0.407	0.300	0.51	0.28	0.385	0.165	0.58	0.32
9	0.400	0.170	0.88	0.34	0.175	0.120	0.19	0.14	0.180	0.130	0.22	0.14
10	0.425	0.410	0.56	0.58	0.287	0.145	0.26	0.14	0.217	0.082	0.26	0.12
11	0.435	0.430			0.410	0.360			0.392	0.197	0.58	0.34
Mean	0.398	0.367	0.71	0.64	0.304	0.213	0.40	0.28	0.246	0.137	0.35	0.19
Standard deviation	0.0539	0.0906	0.147	0.176	0.085	0.080	0.160	0.165	0.0933	0.0469	0.171	0.035
Mean difference	0.031		0.07		0.091		0.12		0.109		0.16	
Standard deviation	0.2250		0.181		0.055		0.165		0.0624		0.079	
Standard error	0.0714		0.060		0.017		0.028		0.0200		0.026	
Probability	>0.6		>0.1		<0.001		<0.01		<0.001		<0.001	
Dogs with hepatic necrosis												
1	0.185	0.220	0.28	0.38	0.210	0.180	0.35	0.32				
2	0.405	0.405	0.45	0.52	0.262	0.255	0.28	0.27	0.160	0.125	0.16	0.12
5	0.335	0.390	0.26	0.39	0.230	0.240	0.18	0.20	0.180	0.130	0.13	0.08
Mean	0.308	0.338	0.33	0.43	0.234	0.225	0.27	0.26	0.170	0.128	0.15	0.110
Mean difference	−0.030		−0.10		0.009		0.01		0.042		0.05	

TABLE II

Lipoprotein lipase levels in four vascular beds in normal dogs

Normal dogs		Change in optical density				Glycerol production			
Subject No.	Post-heparin sampling time	Femoral vein	Femoral artery	Portal vein	Hepatic vein	Femoral vein	Femoral artery	Portal vein	Hepatic vein
	min.		*units*				*μMole/ml.*		
8	10	0.425	0.440	0.430	0.420	0.78	0.74	0.72	0.78
	60	0.407	0.300	0.345	0.140	0.51	0.48	0.49	0.28
	90	0.385	0.320	0.350	0.165	0.58	0.48	0.50	0.32
9	10	0.400	0.320	0.370	0.170	0.88	0.62	0.64	0.34
	60	0.175	0.125	0.155	0.120	0.19	0.24	0.16	0.14
	90	0.180	0.140	0.170	0.130	0.22		0.16	0.14
10	10	0.425	0.420	0.425	0.410	0.56	0.60	0.54	0.58
	60	0.225	0.170	0.225	0.145	0.26	0.22	0.20	0.14
	90	0.147	0.117	0.147	0.082	0.26	0.18	0.22	0.12
11	10	0.435	0.430	0.430	0.430				
	60	0.410	0.410	0.420	0.360				
	90	0.380	0.380	0.392	0.197	0.58	0.58	0.58	0.34
Means for each vascular bed	10	0.421	0.403	0.414	0.357	0.74	0.65	0.63	0.57
	60	0.306	0.251	0.289	0.191	0.32	0.31	0.28	0.19
	90	0.273	0.239	0.263	0.169	0.41	0.41	0.37	0.31

mals was found to be 0.40 μMole in peripheral vein and 0.28 μMole in hepatic vein samples. The mean difference was 0.12 μMole, also significant ($p < 0.01$).

Similar results occurred at 90 minutes post-heparin. The optical density difference was 0.109 unit ($p < 0.001$) and the glycerol difference between peripheral and hepatic vein samples was 0.16 μMole ($p < 0.001$). In one dog 10 mg. of intravenous protamine [3] caused a prompt reduction of lipoprotein lipase activity in both vascular beds. The experimental results with the use of either Nembutal® or ether were similar.

Comparison of lipoprotein lipase in the femoral vein, femoral artery, portal vein and hepatic vein in normal dogs. The question arose: Does the significant difference in the lipoprotein lipase levels of peripheral and hepatic vein blood indicate that the liver removes this enzyme from the blood? The answer could only be in the affirmative if lipoprotein lipase in peripheral vein blood was shown to be equivalent to the level in the blood entering the liver, *i.e.*, by the portal vein and hepatic artery. Such an assumption has appeared true for Bromsulphalein® levels (22). Peripheral vein-hepatic vein differences could mean removal of the enzyme by the lungs, intestinal tract, spleen or kidneys as well as by the liver. These possibilities were investigated in four normal dogs by determining simultaneous lipoprotein lipase levels in the femoral, portal and hepatic veins and the femoral artery. Since hepatic and femoral arterial blood are identical, this experiment permitted a comparison of all blood entering the liver with the blood leaving the liver. Enzymatic activity in blood from these vascular beds and from peripheral venous blood can also be related. As shown in Table II both the optical density change and glycerol production values were much lower in the hepatic vein than in any of the three other vascular beds. At 60 minutes post-heparin, hepatic vein optical density change averaged 0.191 unit as compared with 0.306 unit in the femoral vein, 0.251 unit in the femoral artery and 0.289 unit in the portal vein. The glycerol values from the femoral artery, the femoral vein and the portal vein were grouped together, from 0.32 to 0.28 μMole. These were clearly different from the hepatic vein glycerol production of 0.19 μMole. At 90 minutes post-heparin the data show the same grouping. At 10 minutes post-heparin, as also occurred in the group of normal dogs (Table I), no great differences occurred between any of the vascular beds except for Dog 9 which did have marked differences. In this instance, too, femoral vein, femoral artery and portal vein values were grouped together in contrast to much lower hepatic vein

[3] Protamine sulfate, Eli Lilly and Co.

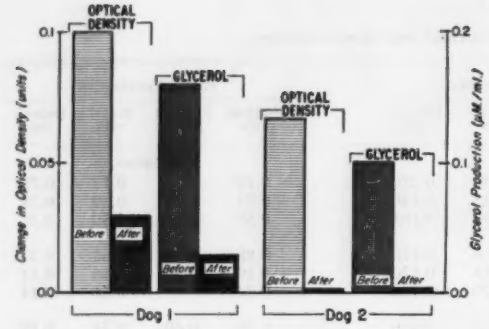

FIG. 1. HEPATIC REMOVAL OF LIPOPROTEIN LIPASE IN TWO DOGS BEFORE AND AFTER DEVELOPMENT OF ACUTE HEPATIC NECROSIS

The peripheral vein–hepatic vein differences for optical density change and glycerol production are given for 60 minute post-heparin specimens.

values for both optical density change and glycerol production. These data fortify the assumption that the lipoprotein lipase level in peripheral vein blood (femoral or antecubital) is representative of the level in the blood entering the liver.

While the lipoprotein lipase levels in the femoral vein and femoral artery are similar, slight differences are apparent in Dogs 8, 9 and 10, but not in Dog 11. Dog 8 at 60 minutes post-heparin had a femoral vein-femoral artery difference of 0.107 optical density unit and 0.03 μMole of glycerol; at 90 minutes differences were 0.065 optical density unit and 0.10 μMole of glycerol. Dog 9 had greatest peripheral venous-arterial differences at the 10 minute time. Dog 10 had differences at 60 and 90 minutes.

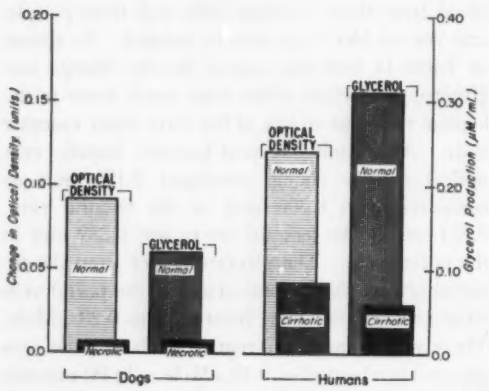

FIG. 2. HEPATIC REMOVAL OF LIPOPROTEIN LIPASE IN DOGS AND HUMANS

Each vertical bar represents the peripheral vein–hepatic vein difference in lipoprotein lipase activity as measured by optical density change and glycerol production. The normal liver is contrasted with the diseased liver in humans having Laennec's cirrhosis and in dogs with acute hepatic necrosis. All values are those obtained 60 minutes post-heparin.

Dogs with hepatic necrosis

Acute liver poisoning with chloroform or carbon tetrachloride greatly reduced the hepatic removal of lipoprotein lipase. At the 10 minute post-heparin time there was a negative peripheral vein-hepatic vein difference of both optical density change and glycerol production (Table I). At 60 minutes post-heparin, the optical density change of the peripheral vein plasma averaged 0.234 optical density unit as compared with the 0.225 unit of hepatic vein plasma. The two vascular beds had also similar glycerol values. At 90 minutes post-heparin there was again little lipoprotein lipase removal by the necrotic liver.

Two dogs, 1 and 2, were studied before and after the induction of acute hepatic necrosis. Figure 1 shows that the normal liver of each dog removed lipoprotein lipase as indicated by peripheral vein-hepatic vein differences in optical density change and glycerol production. After hepatic necrosis there was a great diminution in lipoprotein lipase removal.

A comparison of lipoprotein lipase removal for all dogs indicates greater removal by the normal than by the necrotic liver. Figure 2 compares the mean lipoprotein lipase removal for normal dogs and dogs with hepatic necrosis at 60 minutes post-heparin. The normal dogs had a peripheral vein-hepatic vein difference of 0.091 optical density unit; the dogs with hepatic necrosis had a difference of only 0.009 (one-tenth as much). The normal dogs had a difference of 0.12 μMole of glycerol as compared with only a difference of 0.01 μMole in the dogs with hepatic necrosis. At 90 minutes post-heparin the normal dogs removed 0.109 optical density unit versus a removal of only 0.042 unit for dogs with hepatic necrosis. In terms of glycerol the normal liver removed 0.16 μMole and the necrotic liver only 0.05 μMole.

TABLE III

Lipoprotein lipase in normal humans and in patients with Laennec's cirrhosis

Patient No.	10 min. post-heparin				60 min. post-heparin				90 min. post-heparin			
	Change in optical density		Glycerol production		Change in optical density		Glycerol production		Change in optical density		Glycerol production	
	Peripheral vein	Hepatic vein	Peripheral vein	Hepatic vein	Peripheral vein	Hepatic vein	Peripheral vein	Hepatic vein	Peripheral vein	Hepatic vein	Peripheral vein	Hepatic vein
	units		*μMole/ml.*		*units*		*μMole/ml.*		*units*		*μMole/ml.*	
Normal humans												
1	0.393	0.372	0.96	0.98	0.217	0.055	0.54	0.20				
2	0.380	0.225	0.90	0.62	0.360	0.330	0.96	0.91	0.350	0.270	0.90	0.69
3	0.545	0.470	1.46	0.72	0.338	0.101	0.98	0.56				
4	0.410	0.390	0.96	0.94	0.342	0.202	0.74	0.31	0.292	0.180	0.45	0.20
5	0.270	0.105	0.78	0.31	0.140	0.110						
Mean	0.400	0.312	1.01	0.71	0.280	0.160	0.81	0.50	0.321	0.225	0.68	0.45
Standard deviation	0.0980	0.1459	0.261	0.273	0.0964	0.1091	0.207	0.315				
Mean difference	0.088		0.30		0.120		0.31		0.096		0.23	
Standard deviation	0.0700		0.318		0.0896		0.154					
Standard error	0.0316		0.142		0.0400		0.089					
Probability	<0.05		>0.10		<0.05		<0.05					
Cirrhotics												
6	0.317	0.310	0.92	0.92	0.350	0.347	0.91	0.84	0.340	0.365	0.77	0.79
7	0.545	0.510	0.66	1.00	0.265	0.230	0.35	0.26				
8	0.498	0.475	1.66	1.40	0.405	0.332	1.36	1.12				
9	0.538	0.510	1.22	1.40	0.337	0.315	0.60	0.60				
10	0.480	0.470	0.56	0.72	0.340	0.260	0.52	0.59				
Mean	0.476	0.455	1.01	1.09	0.339	0.297	0.75	0.69	0.340	0.365	0.77	0.79
Standard deviation	0.0927	0.0831	0.447	0.302	0.0173	0.0490	0.402	0.326				
Mean difference	0.021		−0.08		0.042		0.06		−0.025		−0.02	
Standard deviation	0.0141		0.227		0.0332		0.117					
Standard error	0.006		0.101		0.0141		0.052					
Probability	<0.05		>0.40		<0.05		>0.30					

Human subjects

Results in five normal human subjects are tabulated individually in Table III. Subjects 2 and 5 had the greatest peripheral vein-hepatic vein differences for both optical density and glycerol at 10 minutes post-heparin, while Subjects 1, 3 and 4 developed greatest differences at 60 minutes post-heparin. These peak differences ranged between 0.140 to 0.237 optical density unit and between 0.28 to 0.47 μMole of glycerol. When the data from all five normal subjects are considered for the 60 minute post-heparin time, the mean differences were 0.120 optical density unit and 0.31 μMole of glycerol. These were both significant ($p < 0.05$). The mean differences for all subjects at 10 minutes post-heparin were 0.087 optical density unit and 0.30 μMole of glycerol. The two subjects, 2 and 4, studied at 90 minutes post-heparin both showed lipoprotein lipase removal by the liver. Their mean differences were 0.096 optical density unit and 0.23 μMole of glycerol.

The five patients with Laennec's cirrhosis had less clear cut peripheral vein-hepatic vein differences (Table III). Differences were especially minimal as regards glycerol production. The peak difference for each patient occurred at 60 minutes post-heparin and ranged from 0.003 to 0.08 optical density unit and from 0 to 0.24 μMole of glycerol. The mean differences were 0.042 optical density unit and 0.06 μMole of glycerol. The differences at 10 and 90 minutes post-heparin were much less. The one patient studied at 90 minutes had negative differences. The intravenous injection of 10 mg. of protamine in two patients caused the lipoprotein lipase levels in peripheral and hepatic veins to fall to the preheparin levels within 10 minutes.

When the hepatic removal of lipoprotein lipase was compared in normal and cirrhotic subjects, the normal liver removed more lipoprotein lipase (Figure 2). At 10 minutes post-heparin the standard error of the mean differences (normals as opposed to cirrhotics) was 0.0565 optical density unit ($p > 0.05$) and 0.309 μMole of glycerol ($p > 0.05$). At 60 minutes the standard error was ±0.075 optical density unit ($p > 0.1$) and 0.152 μMole of glycerol ($p < 0.05$).

In two normal humans and in two cirrhotic patients the lipoprotein lipase level was higher at 60 minutes post-heparin in the perhipheral vein than in the peripheral artery. The two normal subjects had 15 and 12 per cent higher optical density values in venous samples; the two cirrhotics had 9 and 7 per cent higher values in venous samples. At 10 minutes post-heparin the normal subjects had 5 and 25 per cent higher venous values, but the cirrhotics had no peripheral venous-arterial difference at this time. In terms of glycerol, there were similar venous-arterial differences for the four subjects.

DISCUSSION

These results indicate that the normal liver removes lipoprotein lipase from the blood in both dogs and humans. Another interpretation of the data might be that the liver adds a substance to hepatic vein blood which inhibits the action of lipoprotein lipase. No information is available on this question. The hepatic removal may be regarded as an extraction or denaturation phenomenon in which the lipoprotein lipase content of blood entering the liver is related to the content in blood leaving the liver. The close similarity of the levels of enzyme in peripheral vein blood and in blood entering the liver has been demonstrated. Lipoprotein lipase has been quantified by the change in the optical density of the plasma-coconut oil emulsion which occurred as particle size was reduced and by the production of glycerol from hydrolysis of lipoprotein triglycerides. Both methods of measurement, the optical and the chemical, yielded data supporting the same conclusion about hepatic removal of lipoprotein lipase. A double check on the data was thus obtained. A third technique of measurement, used by others (23), has been the determination of fatty acids formed by enzymatic activity.

If the normal liver removes lipoprotein lipase, then the diseased liver might well have a reduction of this function. Such has been the case in human cirrhosis and in dogs with acute hepatic necrosis. The differences across the liver for optical density change and glycerol production were reduced strikingly. The failure of the diseased liver to perform as did the normal liver provided further evidence of hepatic removal of lipoprotein lipase from the blood. Two reasons

may be advanced for the reduced removal of the enzyme in liver disease. First, hepatocellular damage might prevent a normal function of the liver; second, it is known that anastomoses develop between the portal and hepatic veins in cirrhosis and following hepatic necrosis (24). Such shunts would greatly reduce the usual flow of blood reaching hepatic cells and thus cause an overall impairment of hepatic function.

Previous work on this subject has been done only with the rat, an animal whose liver is said not to contain heparin or mast cells (25). Jeffries (10) and Spitzer and Spitzer (11) found that the isolated perfused rat liver destroyed clearing factor. This was not confirmed by Morris and French who employed a similar preparation (12). We suggest a possible explanation for this one divergent experimental report. The amount of plasma lipoprotein lipase with which the liver is presented may be a critical factor in the determination of hepatic removal by perfusion or sampling techniques. The time of sampling may be another factor. We routinely sampled at several different times after the administration of heparin—at 10, 60 and usually 90 minutes. Heparin effect was maximal at 10 minutes, as indicated by prolonged whole blood clotting times and high levels of lipoprotein lipase. Differences between peripheral vein and hepatic vein levels were then less significant. We might ascribe this lack of difference to the possible overloading of the liver by large amounts of the enzyme. This effect might be expected soon after heparin injection when circulating blood levels of heparin were high and continuing to stimulate the entry of lipoprotein lipase into the blood. For most of the human subjects and for nine dogs the sample times giving the greatest differences were at 60 and 90 minutes when the whole blood clotting times were lower or even normal and overall lipoprotein lipase levels were somewhat lower.

Recently, Robinson and Harris have shown that after a single passage of blood containing heparin through the rabbit hind limb that lipoprotein lipase activity can be detected in the venous return (26). Such a rapid release suggested to them that a lipolytic enzyme was probably derived from the inner lining of blood vessel walls. This work substantiates another report, that the peripheral tissues in the rat release lipoprotein lipase into the blood (10). In humans and in dogs this same enzymatic release by the peripheral tissues appears to occur. We found higher levels of lipoprotein lipase in peripheral venous than in peripheral arterial blood. The high content of lipoprotein lipase in adipose tissue (3) may mean that this tissue or its capillaries supplied at least some of the lipoprotein lipase appearing in the blood after heparin.

The physiological significance of hepatic removal of lipoprotein lipase is uncertain. The enzyme may possibly be destroyed in the liver by heparinase, known to be present in the livers of some species (27). The incubation of lipoprotein lipase with heparinase from liver (11), with a bacterial heparinase (6) and with a liver tissue homogenate (28) all caused a great reduction of enzymatic activity. A recent study demonstrated that injected heparin is inactivated by the liver (29). Heparin or a heparin-like substance appears to be an integral part of the lipoprotein lipase molecule. When heparin was removed from it by passage through an anion exchange resin, enzymatic activity disappeared (30). It could be restored by the addition of heparin. Other evidence indicates that lipoprotein lipase represents a combination of heparin and apo-enzyme from which heparin is dissociated readily (31). Adding further to the idea of the close relationship of heparin and lipoprotein lipase is the often repeated observation that heparin antagonists block the action of the enzyme both *in vitro* and *in vivo*. The prompt decline of lipoprotein lipase levels in the blood following intravenous protamine in our experiments illustrates this antagonism.

Much of the current interest in lipoprotein lipase relates to its possible role in atherosclerosis. It has been suggested that alimentary hyperlipemia or chylomicronemia may be an etiological factor in the development of this disease (32). The delayed clearing of dietary triglycerides in patients with coronary atherosclerosis (33, 34) provides evidence of a possible lipoprotein lipase deficiency in such individuals. Block, Mann and Barker have shown that a small dose of intravenous heparin produced less clearing of the lipemia induced by a fatty meal in coronary patients than in normal persons (35). Others have found that plasma

from atherosclerotic individuals was inhibitory to clearing activity (36, 37). It has been shown, also, that the cofactor activity in the lipoprotein lipase system is reduced in atherosclerotic patients (38).

Patients with cirrhosis of the liver appear to have accelerated clearing of triglycerides from the blood (39) and exhibit very high levels of clearing factor after the injection of heparin (14). Our studies in cirrhotic patients and in dogs with hepatic necrosis suggest that such phenomena may occur because of the reduced removal of lipoprotein lipase from the blood in liver disease. The pathological and clinical evidence indicates that patients with cirrhosis of the liver have less atherosclerosis and myocardial infarction than comparable groups of patients dying from other causes (40-42). One may then pose the question: Might the decreased hepatic removal of lipoprotein lipase in cirrhosis contribute to a lessened incidence of atherosclerosis? The hyperestrogenism, absence of hypertension, hypocholesterolemia, poor nutrition, hypoprothrombinemia, and increased fibrinolysis, all occurring at times in advanced cirrhosis, might be additional contributing factors.

SUMMARY

1. The lipoprotein lipase content of plasma after the administration of heparin was compared in blood collected simultaneously from the peripheral and hepatic veins. In five normal human subjects and in 10 normal dogs the liver removed or inactivated significant amounts of the lipoprotein lipase with which it was presented.

2. Impairment of liver function greatly reduced the hepatic removal of the enzyme. This was shown in five human patients with Laennec's cirrhosis of the liver and in three dogs with acute hepatic necrosis.

3. In four normal dogs the lipoprotein lipase level in the blood entering the liver was similar to the peripheral venous level and different from the hepatic venous level. On the basis of these data hepatic removal of the enzyme could be determined from peripheral and hepatic venous blood.

4. Lipoprotein lipase was measured in peripheral venous and arterial blood in four normal dogs and in four humans. The differences between peripheral venous and arterial blood were consistent with the release of lipoprotein lipase into the peripheral tissues.

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THE NEURAMINIC ACID CONTENT OF CEREBROSPINAL FLUID AS AFFECTED BY NEUROLOGICAL DISEASES *

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Interest in the presence of neuraminic acid in human cerebrospinal fluid (CSF) and the variations in its concentration is derived from the possibility that these may reflect some aspect of central nervous metabolic activity. This, in turn, is based on the fact that neuraminic acid constitutes a part of the molecular structure of those water soluble glycolipids of brain that are generally referred to as gangliosides (1). However, the ubiquitous nature of neuraminic acid in all tissue fluids and structures renders any inquiry based on analysis of CSF for neuraminic acid alone of doubtful significance. It need only be pointed out that neuraminic acid is found in many tissues either as part of the tissue gangliosides or else in mucoprotein-bound forms in such diverse structures as submaxillary glands (2, 3), urinary mucins (4), human serum (5, 6), red cell stroma (1, 7, 8) and even the somatic glycolipid of *Escherichia coli* (9). Hence, in assessing the significance of changes in the neuraminic acid content of CSF, the major problem resolves itself into excluding the neuraminic acid contributed by the serum to the CSF as well as that moiety derived from cellular elements (white and red cells) extrinsic to the central nervous system.

The discovery by Böhm, Dauber and Baumeister (5, 10) that serum contains neuraminic acid (NA) in protein-bound form was followed by the demonstration by Uzman and Rosen (11) that the maximal concentration of NA occurred in the glyco-proteins of the α_1- and α_2-globulin group, with a preponderance in the latter. This was confirmed by the studies of Baudouin, Lewin and Hillion (12), Jayle (13) and others. Since the total NA in serum is almost entirely in nondialyzable form and is almost completely accounted for by the fraction precipitated by trichloracetic acid (14, 15), the methods initially used for the analysis of NA in CSF were adaptations of those used on serum and thus designed to give values only for the protein-bound forms of NA in the CSF. Consequently, early investigations confined to protein-bound fractions precipitated by ethanol and trichloracetic acid (16) disclosed the presence of only this form in CSF, levels of 2.4 μg. per ml. CSF being reported. Subsequent studies on protein-bound NA in the CSF (6) indicated "normal" protein-bound NA levels of 1.4 μg. per ml., although levels reaching as high as 122 μg. per ml. were also observed in cases of meningitis where the CSF protein levels were proportionately elevated. Further studies by Ross and Böhm (17) on the protein-bound NA suggested a "normal" value of 2.7 ± 0.55 μg. per ml. CSF. Although a great variety of neurological diseases was studied by these authors, no pathological process could be correlated with any specific change in protein-bound NA levels. Any process which would be expected to give a rise of the CSF protein concentration also produced a rise in the protein-bound NA concentration.

This picture was modified when studies from this laboratory established that the NA in the CSF is present in two distinct forms (14). The first form is represented by the fraction which is nondialyzable and partly precipitated by trichloracetic acid. This corresponds to the protein-bound forms studied by Böhm, Baumeister and Ross (6, 17). The second fraction is the freely dialyzable NA moiety of CSF. This fraction was shown to account for 60 to 80 per cent of the total NA content of normal human CSF. Since a similar fraction does not appear to occur in demonstrable amounts in serum it was suggested that this freely dialyzable NA fraction may be derived from the metabolic activity of the central nervous

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system. The present study constitutes an inquiry into the significance of variations of NA concentration of the CSF in different neurological diseases, especially with respect to the freely dialyzable NA fraction. Also, by a study of the differences of NA concentration (dialyzable and protein-bound) in ventricular and spinal fluid in cases of known types of pathology, the site of production of the dialyzable NA fraction and the pathological conditions that provide the factors necessary to induce changes in the concentration of this fraction are defined.

MATERIALS AND METHODS

CSF samples were obtained in the course of routine diagnostic lumbar puncture, pneumoencephalography or myelography. Ventricular fluid samples were obtained either in the course of diagnostic ventriculography or by direct puncture through the frontal fontanelle in cases of hydrocephaly or Arnold-Chiari syndrome. In a few instances ventricular fluid was collected from an inlying catheter in patients in whom ventricular drainage was performed. Such samples will be designated hereafter as "drainage samples." For the study of the variation of NA in different parts of the subarachnoid space, a lumbar puncture was performed with the patient in sitting position. As soon as it was ascertained that the puncture was nontraumatic, spinal fluid was allowed to flow out under its own pressure directly into clean collection tubes. Three or four samples, each exactly of 5.0 ml. volume, were sequentially collected. One ml. of each sample was used for total protein estimation by the method employed previously (14) while 3.0 ml. was taken for NA analysis. In those cases where ventricular and spinal puncture were performed simultaneously the ventricular fluid was withdrawn immediately before fluid was allowed to run out of the needle in the lumbar subarachnoid space so that the initial composition of the ventricular fluid was not disturbed.

Neuraminic acid estimation. The analytical procedures were essentially those previously employed (14). The analysis was performed usually on 3.0 ml. of CSF directly, using the procedure of Böhm and colleagues (5, 6) with Bial's reagent. Where NA in protein precipitates was analyzed, the precipitated samples were made up to 1.0 ml. with water and the whole procedure modified accordingly. In some instances the direct reaction with Ehrlich's reagent, as described by Werner and Odin (18), was used to check the quantitative partition of NA in the protein-bound and freely dialyzable fractions. Since, however, the direct reaction with Ehrlich's reagent lacks the desired specificity in spite of extreme sensitivity, the method was used only to corroborate the NA content obtained with Bial's reagent. The main source of error in direct Ehrlich reaction is the color given by α-pyrrole carboxylic acid (19). Total NA values were obtained by treating a 3.0 ml. sample of CSF with 3.0 ml. Bial's reagent (5) in a boiling water bath for 15 minutes. The tubes were cooled and the resulting color extracted into 3.0 ml. of *n*-amyl alcohol, by shaking and centrifugation. The amyl alcohol layer was removed with a capillary pipette, and the absorption of the gray-lavender color compared at 570 mμ against a similarly treated 3.0 ml. water blank. The amount of NA was calculated from the extinction of a standard curve obtained with pure methoxy-neuraminic acid isolated from serum. Duplicates agreed within 1 per cent. The value for total NA thus obtained was corrected for glucose content of the sample whenever necessary as described in the next section.

Since the identity of serum and CSF neuraminic acid has not yet been unequivocally established as being solely represented by the *n*-acetyl derivative, and because the majority of values in the literature are reported essentially as methoxy-neuraminic acid equivalents, the results of our study are also presented in this manner. Thus all NA values reported here are "methoxy-neuraminic acid equivalents."

The methoxy-NA preparation used for the standard curve was a crystalline product isolated from serum (10). It migrated as a single substance in the chromatographic systems described by Klenk, Faillard and Lempfrid (4). The molar ratio of α-amino nitrogen to carboxyl groups (titration) was 1.01. Analysis gave:

Found	C = 42.44	H = 6.80	N = 4.50
Expected for $C_{11}H_{21}O_9N$	C = 42.67	H = 6.96	N = 4.48

Comparison of the color value of methoxy-NA with Bial's reagent and that of crystalline *n*-acetyl-NA [1] obtained from brain strandin (a ganglioside) showed that the *n*-acetyl derivative gave only 73.9 per cent of the color obtained with an equal amount (by weight) of methoxy-NA over a wide range of concentrations tested. Using this conversion factor (100/73.9), the values presented in this study can be converted easily to *n*-acetyl-NA equivalents should future studies prove that all of the protein-bound and dialyzable NA fractions of CSF are identical with *n*-acetyl neuraminic acid.

Protein-bound NA precipitated with trichloracetic acid (TCA-pptd. NA) was determined by treating 3.0 ml. of CSF with 3.0 ml. of cold 10 per cent trichloracetic acid. The precipitate was allowed to collect in the refrigerator for 30 minutes. The supernatant was discarded after centrifugation. The precipitate was next suspended in 1.0 ml. water and treated with 1.0 ml. Bial's reagent in

[1] Kindly provided by Dr. J. Folch-Pi. The analytical authentication of this preparation by Dr. J. Folch-Pi was confirmed by analysis and X-ray diffraction patterns by Professor Blix and established as being identical with *n*-acetyl-neuraminic acid of ovine submaxillary mucin. Detailed information on this preparation has recently been published (Folch-Pi, J., and Lees, M. Studies on the brain ganglioside strandin in normal brain and in Tay-Sach's disease. J. Dis. Child. 1959, **97**, 730).

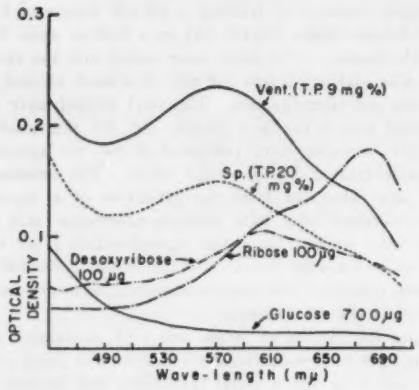

FIG. 1. ABSORPTION SPECTRA OF COLORED REACTION PRODUCTS OF CSF, GLUCOSE, RIBOSE AND DESOXYRIBOSE WITH BIAL'S REAGENT

Note that absorption at 570 mμ of NA in ventricular fluid is much higher than that of spinal fluid even though the former has a much lower protein content (9 mg. per cent). The nonspecific absorption of desoxyribose and ribose at 570 mμ invalidates NA determinations in CSF containing large numbers of white cells.

a boiling water bath for 15 minutes. A blank containing only water (1.0 ml.) and Bial's reagent was similarly treated. The color was extracted into 3.0 ml. *n*-amyl alcohol and the NA content determined from the extinction at 570 mμ. Duplicates agreed within 3.0 per cent.

Protein-bound nondialyzable NA (nondialyzable NA) was estimated by dialyzing 3.0 ml. of CSF for 18 hours against water in a viscose dialysis bag. The contents were quantitatively recovered (with distilled water washings) and made up to 5.0 ml. volume; 3.0 ml. aliquots were used for the estimation of total NA as described above. The calculated nondialyzable NA values agreed within 5 per cent in duplicate determination.

Specificity of the method and correction for glucose content. In order to eliminate the possibility that substances other than NA and its chromogenic derivatives were also being measured with Bial's reagent, the following substances were tested both as aqueous solutions individually and after adding to samples of CSF appropriate amounts of glucose, galactose, D-glucosamine, *n*-acetyl-D-glucosamine, D-arabinose, D-galacturonic acid, α-methyl-D-glucoside tetra-acetate, *i*-erythritol, tetra-acetyl-D-glucose, D-ribose, 2-desoxy-D-ribose, lactose, pyruvic acid and oxaloacetic acid. The hexoses and their derivatives yielded a pale yellow-orange product which, extracted into *n*-amyl alcohol, showed no specific absorption peak at 570 mμ but had greater absorption below 440 mμ (Figure 1). The pentoses on the other hand showed (in 100 μg. per ml. concentration) a very significant absorption at 570 mμ even though the absorption peak of the blue color itself was at 690 mμ for ribose and 600 mμ for desoxyribose (Figure 1). Since sedoheptulose is known to be the only other subtance which produces a specific absorption peak at 570 mμ with Bial's reagent (20), this was also tested for in a number of samples by the independent cysteine-sulfuric acid reaction (20) and its absence was established. Pyruvic and oxaloacetic acids produced no color.

Although glucose has no specific absorption at 570 mμ, the normally high concentration of glucose in the CSF made it necessary to correct for the nonspecific absorption of the yellow-gray color produced with Bial's reagent in the determination of total NA. The interference was negligible in the estimations of nondialyzable or TCA-precipitated NA. The interference was especially marked when the glucose concentration in the CSF was above 40 mg. per 100 ml. This interference can be detected in any given sample by measuring the optical density of the test solution both at 490 mμ and at 570 mμ. Whenever the ratio $E_{490\ m\mu}/E_{570\ m\mu}$ exceeded 0.76 the total NA value had to be corrected for glucose. This was done by an independent analysis of CSF glucose content. This value was read off a glucose curve obtained with Bial's reagent under the same conditions used in the NA analysis to give the Bial color equivalent of the glucose in that particular sample. The contribution of the glucose content to the NA value was subtracted from the total Bial color density to give the true total NA content of the sample (Figure 2).

After completion of this study we were informed by Dr. Eli Robins (21) that ascorbic acid gives sufficient color with Bial's reagent to cause some possible inter-

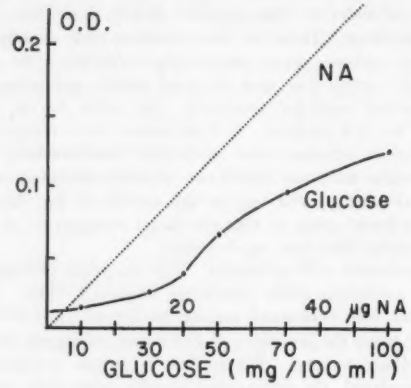

FIG. 2. CORRECTION FOR GLUCOSE CONTENT IN DETERMINATION OF TOTAL NA

The glucose concentration (mg. per 100 ml. CSF) of the sample, read on the abscissa, is related to the corresponding glucose Bial color in a 3.0 ml. CSF sample, by reading off the Bial glucose curve (solid line). This is converted to NA equivalents by reading the corresponding value off the NA curve (dotted line). The figure thus obtained gives the amount of "false" NA which has to be subtracted from the total NA to give the actual total NA content in 3.0 ml. of CSF.

ference in the estimation of total NA in the CSF. We have explored this possibility using a wide range of ascorbic acid concentrations. It was found that up to concentrations of 0.4 mg. per ml. the reaction product with Bial's reagent was pink (maximum absorption at 550 mμ). This changed to brown-green (maximum at 590 mμ) at 1.2 mg. per ml. ascorbic acid concentration. The lowest concentration of ascorbic acid which produced an interference (0.15 mg. per ml.) was found to represent about 10 times the natural concentration of ascorbic acid in CSF. It seems therefore unlikely that this could prove a source of error.

RESULTS

Total NA in CSF samples. A survey of some further 250 samples of CSF conforming to the criteria delineated above (no pleocytosis, no red cells) but containing a wide spectrum of total protein, confirmed our previous report (14) that the total NA content of CSF is largely dependent on the total protein content, especially at elevated protein levels (Figure 3). However, at normal total protein levels (below 30 mg. per 100 ml. CSF) the spread in total NA was quite wide. At this level of total protein the contribution of the dialyzable NA to the total NA level was considerable (40 to 60 per cent) and hence these variations were more easily reflected in the form in which they are expressed (NA as per cent total protein) on logarithmic coordinates for correction of the factor of protein-bound NA. Before any interpretation of the level of dialyzable NA in CSF could be made, it was essential to ascertain whether the dialyzable fraction varied in concentration in the same individual according to the site from which fluid was removed. In other words, although CSF total protein content shows an exponential gradient of increasing concentration from the ventricular reservoir to the lumbar subarachnoid sac, one had to determine whether the total NA concentration maintained the same relationship to the protein at different levels in the subarachnoid space.

Changing concentration of NA in ventricular and spinal subarachnoid spaces. The problem of establishing whether the NA content showed an orderly change in concentration, depending on its concentration at different levels from the lateral ventricles to the lumbar subarachnoid space, was first approached by sequential sampling of CSF (5 ml. aliquots) from the same individual under

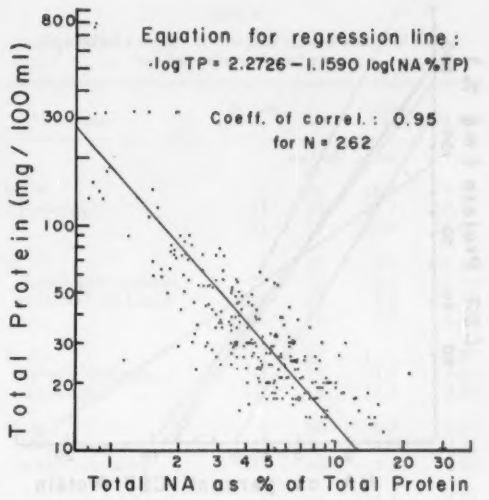

FIG. 3. RELATIONSHIP OF CSF TOTAL NA LEVELS TO TOTAL PROTEIN

conditions in which any disturbance of concentration gradients by manipulations on the examiner's or the patient's part were avoided. Any changes induced by the examiner, such as exerting negative pressure on the syringe (aspirating) or collecting *different* volumes in three or more tubes in sequence were avoided. Similarly, samples collected while the patient moved, coughed, sneezed, struggled or yawned during the procedure invalidated the inquiry, as will be shown. Samples thus collected and analyzed for total protein and total NA revealed as expected that the dialyzable NA concentrations rose exponentially so that the values of the total NA expressed as per cent of total protein and plotted on logarithmic coordinates fell exactly on a straight line (Figure 4). That this phenomenon depended on the maintenance of the natural exponentially-increasing concentration gradient for CSF protein (and protein-bound NA) and the exponentially-decreasing concentration gradient for dialyzable NA was clearly demonstrated when 5.0 ml. samples were removed sequentially from subjects, but 5.0 ml. of air was injected before the next 5.0 ml. sample was collected (Figure 5). In these cases, although the same straight line trend was obvious, the points no longer fell exactly on a straight line. The dependence of unbound NA concentration on the

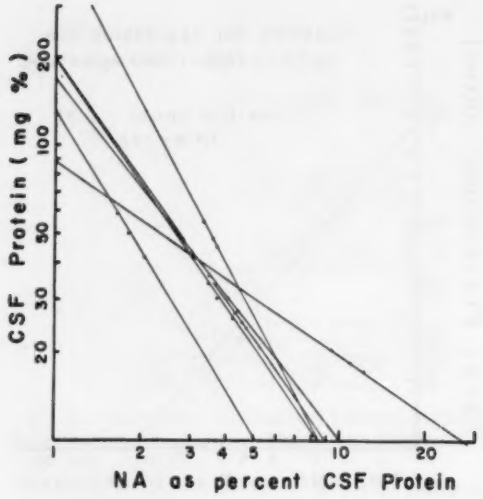

FIG. 4. EXPONENTIAL DECREASE OF TOTAL PROTEIN AND EXPONENTIAL INCREASE OF DIALYZABLE NA MOIETY OF CSF IN SEQUENTIALLY REMOVED EQUAL VOLUMES (5.0 ML.) OF CSF

In each case the first sample has the highest protein concentration. When the normal concentration gradients are not disturbed the values fall on an exact straight line when exponential coordinates are used.

volume of CSF removed from the lumbar subarachnoid space was also evident when the samples removed in sequence from different patients were of unequal volume. As seen in Table I, the total NA content of successive aliquots removed from the lumbar subarachnoid space still constituted a successively larger percentage of the total protein which itself decreased as CSF, originally higher up in the subarachnoid space, reached the withdrawal site. Yet a straight line relationship was no longer evident because of the unequal volumes of the samples.

Simultaneous ventricular-spinal punctures. To confirm the changes in NA concentration observed on sequential removal of equal aliquots of CSF in a direct manner, simultaneous ventricular and spinal taps were performed with the patient lying on his side in six instances. These observations are recorded in Table II. Wide differences in the NA content of ventricular and spinal fluids were in evidence whenever there was some block at the outlet of the ventricular system (Table II, Cases 1 and 2). Although the protein-bound NA was higher in the spinal fluid than in the ventricular cavity, the dialyzable NA moiety was much higher within the ventricular than within the spinal subarachnoid space, as evidenced by a much higher total NA per cent total protein value. This was also true in one case (No. 6) where such a block to the CSF circulation could not be demonstrated. On the other hand, the cases showing a resorptive defect (Nos. 3 and 4) and decreased ventricular NA production (No. 5) showed an equalization of the NA/total protein ratio in the ventricular and spinal fluids.

Variations in NA concentration in the ventricle in pathological states. In view of the foregoing results it would be expected that in internal hydrocephalus, with block either at the level of the aqueduct or the fourth ventricle (noncommunicating hydrocephalus), the content of dialyzable NA would be very high. That such is indeed the case was shown by study of the composition of ventricular fluid from a number of infants in whom total obstruction of the fourth ventricle could be demonstrated. The samples were obtained either by ventricular tap or by continuous drainage (Table III). In six cases of noncommunicating hydrocephalus the average NA content was 27.1 μg. per ml. fluid even though in five of these cases

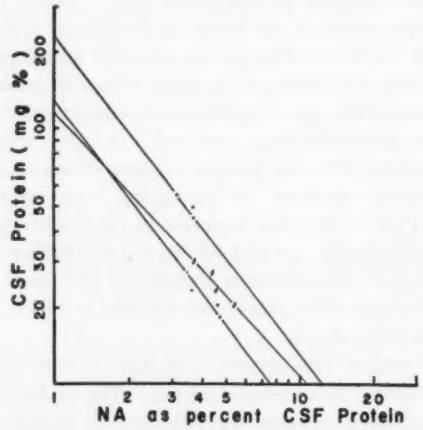

FIG. 5. EFFECT OF DELIBERATE DISTURBANCE OF CONCENTRATION GRADIENTS

Sequentially removed equal volume samples of CSF where the concentration gradients for total protein and NA have been deliberately disturbed by the injection of 5.0 ml. of air between each sample withdrawal.

the total protein content was low. Consequently the contribution of the TCA-precipitated NA to the total was very low too. The exception to this was the observation on the hydrocephalic twins (S.P. and L.P.) in whom a high total NA content was accompanied by a high protein level. These infants had noncommunicating hydrocephalus secondary to severe malformations of the proencephalon and mesencephalon. The atresia of the aqueductus Sylvii was confirmed at autopsy some time later, as communicated to one of us by Dr. Philip Dodge. The observations on cases of noncommunicating hydrocephalus contrasted strikingly with the findings in those cases in which it could be definitely established that the internal hydrocephalus was a communicating one, either by air studies or by the passage of phenolsulfonphthalein from the ventricular to the spinal subarachnoid space. The ventricular total NA content was significantly lower in these patients (average, 17.9 μg. per ml.) even though in all these cases the ventricular protein (and consequently the TCA-pptd. NA) was very much higher (Table III).

Adult patients who had severe cerebral disease of different types and from whom suitable ventricular fluid samples could be obtained showed a remarkably different picture (Table IV). We have included only those cases in which the diagnosis was unequivocally confirmed at operation or autopsy. These cases fell into two groups. Group A was characterized clinically by evidence of progressive increase in intracranial pressure and cerebral edema, with stupor or coma of at least 48 hours' duration. In none of these patients was there any clear cut lateralization of neurological signs. Group B represented patients over the age of 40 with focal seizures of recent onset, or with dementia and focal signs suggesting the possibility of tumor. These patients, with one exception, were alert though often disoriented. In only one patient (F.D.) in this group was there rapidly developing stupor after an acute head injury, but he was included because at the time of ventriculography evidence for increased intracranial pressure and stupor was of only a few hours' duration.

As is evident from Table IV the total NA content was uniformly very low in Group A (average total NA, 9.5 μg. per ml. CSF). Even in those cases where the protein content was markedly elevated, the protein-bound NA represented a relatively small portion of the total NA concentration, indicating an absolute decrease in the dialyzable NA level as well as in the protein-bound moiety. On the other hand, Group B showed only slightly elevated protein levels, while the average total NA content (17.4 μg. per ml.) was almost double that observed in the ventricular fluid of patients in Group A. The difference between the two groups was further accentuated by the finding that in spite of higher total levels the ventricular fluid in Group B had a much lower protein-bound NA content. In other words, the dialyzable NA was very much higher in Group B than in Group A.

TABLE I

Sequential samples of unequal volume removed from lumbar subarachnoid space

Case	Sample no.	TP*	Total NA†	NA/TP
		mg./100 ml.	*μg./ml.*	*per cent*
M. W.	I	20	18.7	9.35
Epilepsy	II	18	19.7	10.90
	III	17	20.5	12.02
M. I.	I	27	9.4	3.48
Temp. lobe seizures,	II	23	8.4	3.65
postictal, 12 hours	III	20	9.2	4.60
	IV	18	8.5	4.72
B. Sy.	I	17	11.7	6.89
Spastic diplegia,	II	17	11.0	6.48
congenital	III	14	12.2	8.70
H. R.	I	60	9.3	1.55
Hypertensive	II	44	12.0	2.73
encephalopathy	III	40	11.7	2.93
One month later	I	34	11.7	3.44
	II	30	11.1	3.70
	III	26	11.1	4.27
D. B.	I	36	12.5	3.47
Basilar artery insuff.	II	30	12.1	4.03
	III	27	11.5	4.36
F. C.	I	32	19.9	6.22
Basilar artery insuff.	II	30	18.7	6.23
	III	26	19.0	7.31
	IV	23	18.9	8.22
J. O'S.	I	27	11.6	4.30
Middle cerebral insuff.	II	25	11.3	4.52
	III	24	11.3	4.71
	IV	24	12.3	5.12
E. T.	I	55	18.4	3.35
Carotid insuff.	II	48	17.3	3.60
	III	45	16.6	3.69

* Total protein.
† Neuraminic acid.

TABLE II

Results of combined ventricular and spinal taps

Case	Age	Right ventricle			Left ventricle			Spinal fluid		
		TP*	Total NA†	NA/TP	TP	Total NA	NA/TP	TP	Total NA	NA/TP
	yrs.	*mg./100 ml.*	*μg./ml.*	*per cent*	*mg./100 ml.*	*μg./ml.*	*per cent*	*mg./100 ml.*	*μg./ml.*	*per cent*
1. H. D. Hydrocephalus (aqueductal atresia)	5/12	7	7.2	10.30	6.0	6.7	11.20	17	8.46	4.98
2. R. S. Basal arachnoiditis (4th ventricle block)	5/12	25	18.21	7.36	19.0	19.0	10.00	50	18.6	3.72
3. B. McL. Communicating hydrocephalus	3/12	21	14.4	7.00	20.0	16.6	7.85	39	13.5	3.49
4. B. B. Communicating hydrocephalus	4/12	75	17.4	2.33	73.0	23.3	3.19	79	24.2	3.05
5. K. B. Right thalamic hemorrhage	59	69	10.6	1.55	64.0	10.8	1.69	90	18.2	2.02
6. W. O. Wernicke's encephalopathy	46	10	10.9	10.90				17	13.1	7.70

* Total protein.
† Neuraminic acid.

Changes in NA content of spinal subarachnoid fluid. In 108 cases arbitrarily chosen on the basis of a CSF total protein content of 30 mg. per 100 ml. or less, there were 47 samples from patients in whom no neurological disease could be demonstrated. The remaining 61 included samples from patients with chronic degenerative disease (four cases Wilson's disease, three cases Huntington's chorea, four cases presenile dementia, four cases Parkinsonism, two cases Jakob-Creutzfeldt disease, two cases familial spastic paraplegia and two cases primary cerebellar degeneration) and

TABLE III

Neuraminic acid content of ventricular fluid in hydrocephalus

Case	Age	TP*	Total NA†	NA/TP	TCA-pptd. NA‡/tot. NA	Protein-bound NA/TP
	mos.	*mg./100 ml.*	*μg./ml.*	*per cent*	*per cent*	*per cent*
Hydrocephalus (internal, noncommunicating)						
B. Az. Complete block, Arnold-Chiari, meningomyelocoele	9	6	18.2	30.00	6.8	2.06
S. P. Meningomyelocoele, drainage sample	36	22	46.6	21.20	7.3	1.54
B. W. Meningomyelocoele, drainage sample	5	21	19.3	9.10	13.5	1.24
P. M. Meningomyelocoele, tot. block	4	9	27.6	30.90	9.0	2.74
(3 months later)		9	26.3	29.20	0.0	0.00
L. P. Hydrocephalic twins (noncommunicating)	1	57	27.9	4.89	34.0	1.66
S. P. Hydrocephalic twins (noncommunicating)	1	42	19.6	4.33	34.9	1.63
B. D. Aqueductal atresia	9	9	31.5	35.00	11.4	4.00
Hydrocephalus (communicating)						
B. T. Hydrocephalus, unknown cause	5	30	19.2	6.40	22.0	1.40
(1 week later)		30	19.4	6.47	23.2	1.50
B. McL. Porencephaly	3	21	14.5	7.00	14.3	1.02
Combined tap: Ventr.		20	15.6	7.85	17.8	1.39
Combined tap: Spinal		39	13.5	3.49	35.0	1.21
B. B. Hydrocephalus, unknown cause	4	75	18.5	2.33	27.0	0.67
Combined tap: Ventr.		73	20.4	3.19	26.2	0.73
Combined tap: Spinal		79	24.2	3.05	44.0	1.35

* Total protein.
† Neuraminic acid.
‡ Trichloracetic acid-precipitated neuraminic acid.

TABLE IV

Neuraminic acid content of ventricular fluid in adult tumor suspects

			TP*	Total NA†	NA/TP	TCA-pptd. NA‡/tot. NA	Protein-bound NA‡/TP
			mg./100 ml.	*μg./ml.*	*per cent*	*per cent*	*per cent*
	Group A—Patients in coma or stupor, evidence of increased intracranial pressure, cerebral edema						
K. B.	Right thalamic hemorrhage (no blood in ventricle)	Right	69	10.6	1.55	38.5	0.59
		Left	64	10.8	1.69	39.8	0.67
R. L.	Right frontal metastatic tumor	Right	29	5.7	2.00	30.0	0.59
		Left	24	7.4	3.09		
A. G.	Medulloblastoma with 4th ventricle block		81	16.0	1.86	34.3	0.68
W. L.	Subdural hygroma		29	7.8	2.66	20.5	0.55
F. D.	Dementia, diabetes	Right	156	13.2	0.84		
		Left	142	12.4	0.89		
G. M.	Metastatic adenocarcinoma	Left	132	10.4	0.80		
	(1 week after operation)	Left	132	12.1	0.92		
W. T.	Subdural abscess		15	5.4	3.74	23.2	0.83
			16	6.4	4.12		
			10	5.2	5.40	18.5	0.96
	Group B—Patients with dementia and severe focal signs, but alert, no evidence of increased intracranial pressure						
G. P.	Severe atrophy of right hemisphere	Right	17	11.6	6.94	19.1	1.30
		Left	17	14.2	8.29	16.1	1.34
	(repeated)	Left	29	10.2	3.53	28.4	1.00
H. McK.	Anterior cerebral artery thrombosis	Right	12	19.2	16.25	12.6	2.02
V. F.	Cerebral atrophy, old infarct	Right	25	17.2	6.80	14.2	0.98
		Left	23	15.7	6.80	14.6	0.99
T. B.	Aneurysm, right middle cerebral artery	Right	15	17.4	11.60	16.7	1.93
		Left	19	17.1	10.90	18.1	1.62
W. C.	Wernicke's encephalopathy	Right	10	10.9	10.90	9.17	1.01
J. D.	Alcoholic dementia, focal seizures		24	23.4	9.75	13.67	1.34
J. B.	Dementia, large ventricles, metastatic carcinoma	Right	24	24.0	10.00	16.35	1.63
		Left	24	24.4	10.20	15.02	1.54
F. K.	Acute head trauma, developing stupor and focal signs on right side, aphasia	Right	15	18.6	12.40	10.27	1.27
		Left	12	20.1	16.75	8.95	1.50

* Total protein.
† Neuraminic acid.
‡ Precipitated by trichloracetic acid.

patients with temporal lobe seizures, chronic cerebral vascular disease and evidence of obstruction of the spinal subarachnoid canal (evidence of block at myelography or at operation). As is evident from the histogram in Figure 6, there was a considerable overlap in the distribution patterns of total NA concentration in the patients with and without neurological disease. The range in the "normal" samples was especially wide (8 to 32 μg. per ml. CSF) with a peak between 16 and 20 μg. per ml. Those cases with CSF protein levels below 30 mg. per 100 ml. with evidence of neurological disease had a total NA concentration distribution of 4 to 20 μg. per ml. CSF with a sharp peak between 8 and 14 μg. per ml. When this information was further broken down it was seen that all cases with verified spinal subarachnoid obstruction had total NA values below 10 μg. per ml. CSF. The cases with chronic degenerative

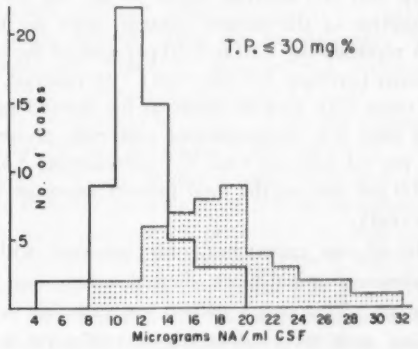

FIG. 6. OVERLAP OF TOTAL NA IN PATIENTS WITH AND WITHOUT NEUROLOGIC DISEASE

Histogram showing the distribution pattern of total NA concentration in 108 cases arbitrarily chosen because the total protein was 30 mg. per cent or less. Stippled area represents normal cases, blank area those with neurological diseases.

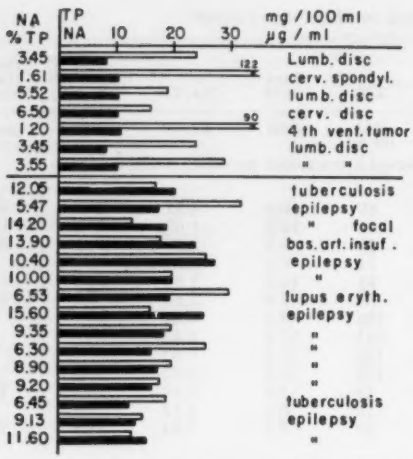

FIG. 7. REDUCTION OF NA IN SPINAL BLOCK

Showing the markedly reduced NA concentration (less than 10 μg. per ml.) in lumbar fluid in cases with proven block above the site of sampling, as compared with patients having other types of neurological disorders. Black bars represent total NA concentration, white bars total protein concentration.

disease and chronic cerebral vascular disease represented those values that directly overlapped the "normal" range, the same being true for those patients with convulsive seizures with or without evidence of focal lesions. The striking reduction in the total NA concentration of the CSF in cases with varying degrees of spinal block is illustrated in Figure 7. The seven cases of verified spinal block had NA content below 10 μg. per ml. irrespective of the protein content, with the total NA representing 1.20 to 6.50 per cent of the total protein (average 3.61 per cent). In contrast, the 15 cases with protein levels in the normal range had total NA concentrations uniformly above 10 μg. per ml. with the total NA representing 5.47 to 15.60 per cent of the total protein (average 9.93 per cent).

In all our experience, only one case without evidence of spinal block showed a very low NA content. This was a patient not included in the tables, with severe dementia and bilateral hemiparesis with spasticity, who was shown both by arteriography and surgical exploration to have a total occlusion of both internal carotid arteries. In this patient the CSF total protein was 21 mg. per 100 ml., the total NA, 9.7 μg. per ml.; NA as per cent of total protein had a value of 4.55, but the TCA-pptd. NA represented 17.1 per cent of the total.

The protein-bound NA fraction. Inasmuch as the relatively small volumes of the CSF samples available restricted the analysis of the protein-bound NA moiety to that obtained from analysis of the TCA-pptd. fraction, it was discovered that the latter did not represent the whole of the protein-bound NA moiety. In 48 cases where adequate volumes of CSF were available and different levels of total protein concentration prevailed, analyses of both TCA-pptd. NA and the nondialyzable NA were performed. In all instances the nondialyzable NA fraction was much greater than the TCA-pptd. NA fraction, the latter usually representing only about 50 per cent of the former (Figure 8). The slope for the regression line for the values plotted in Figure 8 was 0.63, with a standard error about the regression line of 6.73, and a coefficient of correlation "r" equal to 0.86. On the other hand, the nondialyzable NA fraction was directly related to the total protein concentration of the CSF in 48 cases studied (Figure 9). In 46 samples with differing protein content (range, 11 to 90 mg. per 100 ml.), sufficient fluid was available for the estimation of nondialyzable NA as well as for total NA concen-

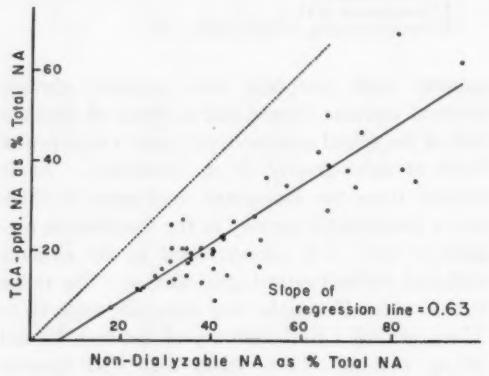

FIG. 8. RELATION OF NONDIALYZABLE NA FRACTION TO TCA-PRECIPITATED FRACTION

Showing that the nondialyzable NA fraction of CSF is not equal to the TCA-precipitated fraction, the latter always being less. Dotted lines represent the line along which the points would have fallen, had the two fractions been equivalent to each other.

tration. The average nondialyzable NA represented 2.0 per cent of the total protein (mean, 2.05 per cent ± 0.14; standard deviation, 0.922 per cent) in these samples with a range of 0.76 to 4.42 per cent. On the other hand the TCA-pptd. NA represented on the average 0.97 per cent of the total protein (mean, 0.966 per cent ± 0.0394; standard deviation, 0.332 per cent) in the CSF from 71 patients with a range of 0.42 to 2.0 per cent, while the total protein concentration ranged from 11 to 275 mg. per cent. In neither case was there any relationship between the protein concentration and the magnitude of the value of bound NA as per cent total protein. In other words, changes in the ratio of protein-bound NA to total protein in the CSF could not be shown to be dependent on changes in protein concentration.

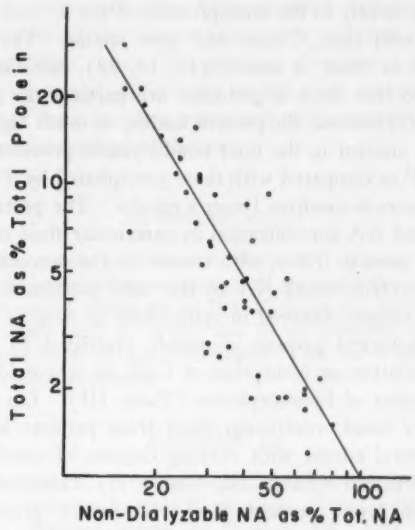

FIG. 9. RELATIONSHIP OF NONDIALYZABLE NA CONCENTRATION TO CSF TOTAL PROTEIN CONCENTRATION

The straight line represents the theoretical curve if all the nondialyzable NA of CSF were derived from serum. Thus, if the nondialyzable NA represented *all* the NA in CSF (100 per cent) then the NA would be 1 per cent of the CSF total protein (the same as in serum).

DISCUSSION

The assessment of the significance of change in concentration of any substance in the CSF requires (*a*) that the changes observed in any pathological process be appreciably beyond the margins imposed by any concentration gradient for that particular substance, and (*b*) that all possible changes in the circulation pattern be accounted for before any changes in concentration are related to the pathological process itself. Data presented in this paper clearly show the existence of a concentration gradient for total NA as well as for the dialyzable NA and the protein-bound NA moieties. There appears to exist an inverse relationship between the concentration gradients of protein-bound NA and dialyzable NA. The latter is highest in the ventricle and decreases sharply in the spinal subarachnoid fluid, while the protein-bound NA is in lowest concentration in the ventricle and increases *pari passu* with the protein concentration as the spinal subarachnoid space is reached. In line with these considerations, changes in the total NA content of CSF occurring with disease processes fall into two categories. The first represents those processes that produce an increase in the total protein concentration of the CSF (irrespective of etiology) and thus produce a rise in the protein-bound NA. In these states the increased NA content is of no greater significance than the nonspecific increase of CSF proteins. This conclusion supports the observations of Ross and Böhm (17). In their limited study of 20 cases, these authors reported that protein-bound NA (measured as TCA-pptd. NA) represented 0.75 to 1.86 per cent of the total protein, with two "normal" values reported as 0.91 and 0.80 per cent of the total protein. The difference found between these TCA-pptd. NA values and those obtained when the nondialyzable NA was analyzed (Figure 8) indicates that, unlike serum, only a portion of the protein-bound NA in CSF is precipitated by TCA. Thus our observations on the nondialyzable NA fraction of CSF obtained from the lumbar subarachnoid space indicate a higher percentage of NA in the protein-bound form (average 2.0 per cent of the total protein, range 0.75 to 4.42 per cent) than in the TCA precipitations by us and by others (16, 17). The reason for this difference probably resides in the fact that the CSF contains about 15 times more proteins *not* precipitated by TCA than does serum, as was recently shown in a detailed study by Bauer (22). The same author showed that those fractions which are not precipitated by TCA be-

long mostly to the mucoproteins of the α_1- and α_2-globulin class. Since our own studies (11) as well as those of others (12, 13, 23), have indicated that these α globulins are particularly rich in NA content, the present finding of much higher NA content in the total nondialyzable proteins of CSF as compared with those precipitated by TCA appears to confirm Bauer's results. The protein-bound NA concentration in ventricular fluid does not seem to differ, with respect to the percentage of protein-bound NA to the total protein, from the ranges observed in spinal fluid as long as the pathological process is mainly restricted to the circulation or resorption of CSF, as was evident in cases of hydrocephalus (Table III). On the other hand, ventricular fluid from patients with cerebral edema with varying degrees of cerebral destruction (Table IV, Group A) exhibited a much lower proportion of NA in the protein-bound fraction. We are inclined to believe that the probable explanation for this is that the increase in ventricular protein in these cases was mainly due to exudation of albumin which is known to be essentially free of neuraminic acid (11, 23).

In contrast to the changes in the total NA content induced by changes in the protein-bound NA, changes in the dialyzable NA moiety appear to be brought about mainly by changes in the circulation pattern of the CSF. The highest values were found in the ventricles where the outflow from the fourth ventricle was totally obstructed by the phenomenon of Arnold-Chiari (Table III) while lesser concentrations of dialyzable NA prevailed when the hydrocephalus was a communicating one. Similarly, drastic decreases of the dialyzable NA concentration were observed in spinal subarachnoid fluid if fluid was obtained below the site (or sites) where evidence of spinal subarachnoid block existed (Figure 7). In many instances this decrease was evident without an increase in the total protein. Although our proven cases of spinal subarachnoid block are too few to justify any categorical conclusions, it does appear that such a decrease of dialyzable NA concentration in the spinal subarachnoid space may serve as a more sensitive index of physiologic block of CSF circulation than the well known elevation of total protein.

Decreases in the concentration of dialyzable NA were also observed in ventricular fluid (Table IV, Group A). The pathological findings were different in every case at surgery or autopsy. The only common denominator in this admittedly small number of cases was coma with evidence of cerebral edema and increased intracranial pressure. The only explanation that suggests itself is that cerebral edema, with its consequent metabolic disturbances and restrictions in extracellular fluid circulation in the cerebral substance, interferes with the production of dialyzable NA into the ventricular cavity. This finding, taken in conjunction with the fact that in every other instance, irrespective of pathology, the dialyzable NA fraction was found in highest concentration within the ventricular cavity, lends further support to our original suggestion (14) ascribing the presence of this fraction to the metabolic activities of the cerebral substance itself.

Our failure to note any differences between the dialyzable NA fraction of CSF in patients with chronic degenerative disease of the nervous system (Wilson's disease, Huntington's chorea, Alzheimer's disease, Jakob-Creutzfeldt disease, primary cerebellar degeneration and so forth) and that of patients without neurological disease does not appear surprising. It seems unlikely that the NA contributed to the ventricular fluid by a chronic degenerative-destructive process could have any significant reflection in the dialyzable NA moiety of the CSF as a whole because of the steep concentration gradients prevailing for this fraction. This last factor would tend to overshadow any small concentration change in the ventricles.

The results obtained on sequential sampling of CSF at the lumbar subarachnoid space (Figure 4) demonstrate a regularity in the normal concentration gradient of the total NA of CSF. Disturbances in this concentration gradient (Figure 5) and the results of sampling of unequal volumes (Table I) clearly validate the extrapolation of the straight line relationship observed under ideal conditions (Figure 4). The intercept of this straight line with the total NA as per cent total protein abscissa represents the theoretical total NA content of the ventricular fluid, if the total protein is approximately 10 mg. per cent. In any

given case the sequential sampling of equal volumes of CSF from the lumbar spinal subarachnoid space and treatment of the data, as illustrated in Figure 4, should prove a very useful way of estimating the approximate total NA concentration in the ventricle. This is especialy evident in the study of cases in which ventricular fluid is not available, and the method consequently deserves further exploration.

SUMMARY

Previous observations on the variations of neuraminic acid concentration of cerebrospinal fluid (CSF) have been extended to 262 cases. From this group, several smaller groups of cases in which the diagnosis was established at surgery or autopsy were chosen to correlate the pathophysiological variations of neuraminic acid (NA) content with specific types of pathological processes in the nervous system.

It was shown that the total NA content of CSF represents the sum of two fractions. One of these is the protein-bound NA fraction which increases in nonspecific manner with increasing CSF protein concentration irrespective of the pathological conditions giving rise to this increase. It is in lowest concentration in ventricular fluid, and increases *pari passu* with the protein concentration in the lumbar subarachnoid fluid. The variations encountered in the relationship of the concentration of this fraction to the total protein concentration appear to reflect the changing proportions of α_1- and α_2-globulin glycoprotein components of the CSF total protein. Unlike its counterpart in the serum the fraction of protein-bound NA precipitated with trichloracetic acid represents only 40 to 60 per cent of the total nondialyzable NA in CSF. The other is the dialyzable NA fraction which is found in highest concentration in the ventricular fluid and decreases exponentially as the lumbar subarachnoid space is reached. This fraction was found to be significantly diminished in ventricular fluid in cases with severe cerebral edema irrespective of etiology. It was also found to be markedly decreased in spinal subarachnoid fluid removed below the site of subarachnoid block, whether the block was at the level of the aqueduct, fourth ventricle or cervical cord. The highest levels were observed in ventricular fluid in cases of noncommunicating hydrocephalus due to obstruction at the level of the fourth ventricle. Patients with chronic degenerative disease exhibited no significant differences in the CSF content of either fraction when compared with patients having no neurological disease.

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THE DISTRIBUTION OF INTRAVENOUSLY-INJECTED INULIN IN THE FLUIDS OF THE NERVOUS SYSTEM OF THE DOG AND RAT *

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In any quantitative study of the degree of damage to the blood-brain barrier, it is necessary to be able to measure the degree of permeability of the barrier to a substance which does not ordinarily pass through it. Bakay (1) employed P^{32} labeled inorganic phosphate for this purpose; he measured the amount of the substance taken up by the brain tissue of cats after injuries which caused damage to the barrier. Unfortunately, once inorganic phosphate passes through the blood-brain barrier, it is rapidly taken up by the nerve cells so that its absolute concentration in the tissue is no accurate measure of the degree of damage to the barrier. For the purpose of measuring the degree of this damage, it would be more advantageous to use a substance which, unlike P^{32} labeled inorganic phosphate, does not enter nerve cells once it has passed through the damaged barrier. Inulin, which has been used extensively in extracellular fluid measurements in other tissues, might serve for such studies. Unfortunately, there is no detailed account of the degree of penetration of nervous tissues by inulin in the living animal. This is surprising in view of the amount of information relating to the distribution of inulin in the fluids of other tissues such as muscle (2–5).

Allen (6) has determined the volume of distribution of inulin in tissue slices of brain by immersing them in solutions of inulin long enough for diffusion to take place and then estimating the inulin content of the slices. He found that about 14.5 per cent of the tissue consisted of fluid to which inulin had access; and, since this value agrees very well with values obtained for the extracellular fluid content of other tissues as measured using inulin (7), it seems reasonable to assume that inulin does not penetrate the cells of the nervous tissue to any greater extent than it penetrates the cells of the rest of the body. Allen's studies (6) did not throw any light on the way in which inulin was handled by the blood-brain barrier, although he remarked in his paper that inulin does not penetrate the blood-brain barrier. In much the same fashion, many others writing on the body fluids have made the statement or assumed that inulin does not enter the cerebrospinal fluid (7, 8). The fact that inulin does not enter the fluids of the gastrointestinal tract has been demonstrated (9); but, apart from an observation made by Swan, Madisso and Pitts in the course of a related study (10), little is recorded of its behavior with respect to fluids of the central nervous system. Substances similar to inulin in chemical composition such as fructose and sucrose have been shown to enter the brain slowly (11), and it might be presumed that inulin behaved in a similar fashion.

In order to explore the possibility of using inulin as a measure of the integrity of the blood-brain barrier and at the same time to obtain information about the nature of the extracellular fluids of the nervous system, the following study of the degree of penetration of inulin into the normal brain and spinal cord of dogs and rats was carried out. It also seemed convenient at this time to find out to what extent inulin entered the cerebrospinal fluid of dogs.

METHODS

The experiments were carried out on mongrel dogs and male rats of the Osborne-Mendel Strain.

The dogs which had been fasted overnight were bilaterally nephrectomized after anaesthesia had been induced with Nembutal® (.5 ml. per Kg. body weight intravenously). After the withdrawal of a control sample

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TABLE I

Relationship between simultaneous extracellular fluid and cerebrospinal fluid inulin levels at end of each experiment on the dogs

Control dogs—no inulin injected

Dog	Weight	Duration of experiment	Inulinoid blank level ECF	Inulinoid blank level CSF
no.	*Kg.*	*hrs.*	*mg./100 ml.*	*mg./100 ml.*
11	10	3	2.1	1.9
7	10	5	2.1	1.6
9	9	5	1.7	1.8

Experimental dogs—inulin injected

Dog	Weight	Duration of experiment	Inulin level ECF*	Inulin level CSF†	Ratio $\frac{\text{Concentration CSF inulin}}{\text{Concentration ECF inulin}}$
no.	*Kg.*	*hrs.*	*mg./100 ml.*	*mg./100 ml.*	
1	7	3	12.0	0.0	0
2	16	3	12.0	0.0	0
3	8	3	21.6	0.0	0
4	10	3	42.8	1.9	.044
10	10	3	55.2	2.0	.036
12	9	3	102.0	2.8	.027
14	8	3	110.0	2.6	.024
8	11	5	29.0	0.1	.003
6	11	5	66.8	2.0	.030
13	11	6	85.5	1.3	.015
15	8	7.5	38.5	2.2	.057

* This represents the difference between the total chromogen level at the end of the experiment and that found before the injection of inulin.

† This represents the difference between the total chromogen level in the CSF of each animal and the average blank value found in the control animal.

of blood, a solution of inulin was infused intravenously, the amount administered to each animal being chosen in such a way that the individual serum inulin levels, after equilibration in body fluids had taken place, were spread between values of 10 mg. per cent and 100 mg. per cent. In three dogs (Table I, Numbers 7, 9 and 11) no inulin was injected but the animals were subjected to all the other procedures.

At intervals varying from three to seven hours after the injections of inulin a sample of cerebrospinal fluid (CSF) was obtained from each dog and immediately following this, blood was obtained by cardiac puncture. The CSF was withdrawn by the introduction of a needle such as is used for lumbar puncture in humans into the cisterna cerebello-medullaris from the back of the neck. After the cardiac puncture each animal was killed by injecting Nembutal® intravenously. Immediately after death in Animals 7 to 14, inclusive, the cranial vault was removed to expose the brain and the spinal cord was displayed by a rapid laminectomy. In each of the dogs dissected in this way, portions of cerebral cortex, white matter and spinal cord were removed, washed rapidly in a dish of 0.15 M saline, dried quickly on filter paper, and placed in tared, stoppered tubes.

The eight male rats studied weighed about 250 Gm. each and were fasted during the night before the experiment but allowed water freely. A bilateral nephrectomy was carried out in each under ether anaesthesia and in six of them 1 ml. of a 2 per cent solution of inulin in 0.15 M saline was injected intravenously. In two of the rats used as controls (Table II, Numbers 247 and 253), no inulin was injected but 1 ml. of the saline solution was used. Three hours after the inulin injection, blood was withdrawn from each rat by aortic puncture under anaesthesia, and the animal was killed. The cranial vault was removed in each animal, a piece of each cerebral hemisphere removed quickly, washed, dried on filter paper, and transferred to a tared, stoppered tube. It was not possible to procure samples of CSF in the rats nor was it easy to discriminate between white and gray matter as was done in the case of the dogs.

Preparation of samples. To a 1 ml. aliquot of serum separated from each blood sample was added 15 ml. of distilled water, 2 ml. of a 0.3 M solution of $Ba(OH)_2$ and 2 ml. of a 5 per cent solution of $Zn(SO_4)_2$. The precipitate was removed by filtration and the resulting solution was analyzed for inulin. The CSF samples were treated similarly. After reweighing the tubes containing the tissues, 3 ml. of distilled water was added to each and the tissue well minced in it with a stirring rod. The resulting suspensions were kept overnight in the refrigerator and 3 ml. of a 20 per cent solution of trichloracetic acid was added to each of them; the resulting precipitate was removed by filtration. Aliquots of the filtrate were taken and analyzed for inulin.

Inulin method. Inulin was determined throughout by

the resorcinol method for fructose of Bacon and Bell (12) as modified for inulin by Higashi and Peters (13).

Depending on whether serum and CSF or tissue was being analyzed for inulin, a suitable standard was used which had been treated in a similar fashion with respect to protein precipitant. The standards were prepared by appropriate dilution of the original inulin solution used for injection.

Recoveries. In order to estimate the sensitivity of the method for tissue inulin, recoveries were carried out. A piece of normal dog spinal cord weighing from 1 to 2 Gm. was placed in each of six tared tubes and reweighed. Each sample was prepared for analysis in the same fashion described above for the tissues from animals. After filtration of the precipitated proteins, separate 2 ml. aliquots of the filtrates were taken and to them were added 2 ml. amounts of water in which had been dissolved 0.005 mg., 0.015 mg., 0.020 mg. and 0.025 mg. amounts of inulin, respectively. The resulting 4 ml. aliquots containing the added inulin were analyzed for inulin, using as a blank a further 2 ml. aliquot of each filtrate to which had been added 2 ml. of distilled water. The recoveries are set out in Table III. It can be seen that, as the added inulin rose above 0.02 mg. per Gm. of tissue, the accuracy of the recovery was more satisfactory, being of the order of 105 per cent. This means that, where the serum inulin level was 20 mg. per cent, an estimation of the inulin space in the nervous tissue could be made if it exceeded 4 per cent of the wet weight of the tissue, with an error of about 5 per cent. Except in the case of Animal 8 (Table I), the serum level in the dogs was at least 50 mg. per cent, thus allowing a greater accuracy in the determination of smaller inulin spaces. It is evident from the recovery figures that there was a tendency to overestimate the inulin content of the tissue and therefore the inulin space.

TABLE II

Extracellular fluid inulin levels and inulin spaces of the nervous tissues in the rats

Control animals—no inulin injected

Rat	Inulinoid blank	
	ECF	Nervous tissue
no.	*mg./100 ml.*	*mg./100 Gm. tissue*
247	1.9	4.6
253	1.7	4.2

Experimental animals—inulin injected

Rat	ECF inulin*	Total inulin chromogen of nervous tissue	Inulin space
no.	*mg./100 ml.*	*mg./100 Gm. tissue*	*Gm./100 Gm. tissue*
246	44.5	5.5	2.5
248	42.5	5.8	3.3
249	56.0	6.2	3.1
251	49.2	6.0	3.3
252	55.6	5.8	2.5

* This represents the difference between the total chromogen level in the experimental animals and the blank chromogen found in the controls.

TABLE III

Recoveries

Tissue	Inulin added	Inulin recovered	Recovery
	mg./Gm. tissue	*mg./Gm. tissue*	*per cent*
1	0.008	0.009	111
2	0.011	0.012	109
3	0.011	0.013	118
4	0.038	0.040	103
5	0.030	0.031	103
6	0.025	0.026	104

Calculations. The difference between the total serum chromogen at the end of the experiment and the inulinoid chromogen blank on the serum drawn at the beginning, before the inulin injection, gave the serum level of the injected inulin. The concentration of inulin in the extracellular fluids (ECF) which was presumably the same as that in serum water was derived from that found in the blood serum by multiplying it by the factor 1.07.

The total chromogen present in each sample of tissue was calculated, taking into account the water content of the tissue which was assumed to be 78 per cent of its total weight (6). From this figure the chromogen content per 100 Gm. wet tissue was obtained. Similar values were obtained in the tissues from the control animals and these figures represented the chromogen blank. By subtracting the average value of the tissue chromogen blank obtained in the control animals from the total chromogen content of each tissue in the experimentals, a figure was obtained which represented the content of injected inulin in each sample of tissue. From the inulin content of the nervous tissue in each animal and its concentration in ECF the inulin space of the tissue was calculated by simple division and expressed as a mass of fluid in grams for unit weight of the tissue.

RESULTS

Serum and cerebrospinal fluid levels

Table I sets out the simultaneous ECF and CSF levels of inulin in each dog. The concentration of ECF inulin has been derived from the serum inulin as described above. In the animals with the higher ECF inulin concentrations there appeared to be slightly higher levels in the CSF, but the relationship was erratic. In Animals 1, 2, 3, 4, 10, 12 and 14, the experiment was continued for three hours and, when there was more than 40 mg. per cent of inulin in the ECF, a slight increase in the CSF inulin was detected; but fur-

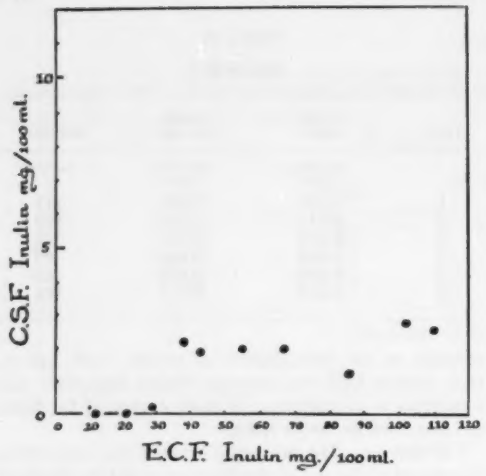

FIG. 1. SIMULTANEOUS CEREBROSPINAL FLUID (CSF) AND EXTRACELLULAR FLUID INULIN (ECF)
Concentrations at the end of each experiment.

ther elevation of the ECF level to values approaching 100 mg. per cent did not seem to produce any significant increase in the concentration in the CSF. Some of these effects may have resulted from overestimation of the inulin at low concentrations due to the relative insensitivity of the method.

In order to test the effect of a more prolonged experiment, the period between the introduction of the inulin and the withdrawal of the CSF was extended as in Animals 6, 8, 13 and 15. Here, although the ECF inulin levels were comparable to those prevailing in the three hour experiments, no striking increases were observed in the inulin concentration of the CSF of the animals. The variation in the ratio $\frac{\text{Concentration of inulin CSF}}{\text{Concentration of inulin ECF}}$ was a further indication of the erratic relationship between the inulin levels of the two fluids. Neither extension of the experimental period nor increase in the serum levels seemed to bring about any consistent changes in the ratio over the range of concentrations studied, although the figures suggest that inulin entered the CSF to a slightly greater degree when the ECF level was higher. The relationship between the level of inulin in the ECF and that in the CSF is set out in graphical form in Figure 1. It is easily seen that when the inulin level of the ECF rose above 30 mg. per cent small but detectable quantities of inulin were present in the CSF. At the levels of ECF inulin studied the concentrations of it in the CSF were certainly very much lower than in the ECF.

Inulin space of the nervous tissues

In Tables IV, V and VI are set out the data pertaining to the inulin space of the spinal cord, white matter and gray matter of each of the dogs studied. The inulinoid blanks found in the same tissues of the control animals (Dogs 7, 9, 11) are at the top of each table. The inulin space in each piece of tissue analyzed has been calculated on the assumption that the average inulinoid value for that particular tissue in the control animals can be used as a chromogen blank to subtract from the total chromogen value in the same tissue of the experimental animal. The inulin space is set out in the right hand column of each table.

It can be seen from Table IV that the inulin space for the spinal cord was, at the most, 2 per cent of the tissue weight. In only one animal (No. 12) did the inulin space exceed that value in both samples of spinal cord and there the inulin space was not greater than 4 per cent of the

TABLE IV

Inulin space of the spinal cord in the dogs

Control animals—no inulin injected

Dog		Inulinoid substance
no.		*mg./100 Gm. spinal cord*
7	Sample A	3.9
	Sample B	3.7
9	Sample A	3.5
	Sample B	2.9
11	Sample A	3.8
	Sample B	3.4

Experimental animals—inulin injected

Dog		Total inulin chromogen	Inulin space
no.		*mg./100 Gm. spinal cord*	*Gm./100 Gm. spinal cord*
8	Sample A	3.5	0.0
	Sample B	4.0	1.8
10	Sample A	3.7	0.4
	Sample B	4.9	2.5
12	Sample A	7.8	4.2
	Sample B	6.9	3.3
13	Sample A	4.7	1.4
	Sample B	4.9	1.6
	Sample C	6.0	2.9
14	Sample A	5.5	1.8
	Sample B	5.7	2.0

weight of the tissue. Values of a similar order of magnitude were found for the inulin spaces in the case of both white and gray matter (Tables V and VI).

If the assumption is made that the amount of inulin in each piece of tissue, and thus the inulin space, was being underestimated by the amount of the inulinoid blank value, then a figure, which represents the extreme limits of such an underestimated inulin space, may be calculated. Even on this basis the inulin space represented only about 5 per cent of the tissue weight and only rarely did it exceed 10 per cent by weight of the tissue.

Comparison of the blank values obtained in the three control animals showed a moderate variation in individual animals (Tables IV, V and VI, Animals 7, 9 and 11). The variation in the different tissues examined was consistent in any one animal, being highest in the cortex and lowest in the spinal cord. The intermediate position of the white matter may be related to the fact that it was virtually impossible to obtain white matter without adherent gray cortex. The extent to which this variation in the blank chromogen value of the different tissues may be due to the proportion of each tissue that was made up of blood from the vascular bed cannot be determined, as no estimation of the amount of blood remaining in the individual tissues was carried out.

TABLE V

Inulin space of cerebral white matter in dogs

Dog		Inulinoid substance
	Control animals—no inulin injected	
no.		*mg./100 Gm. white matter*
7	Sample A	4.4
	Sample B	4.9
	Sample C	4.5
9	Sample A	4.3
	Sample B	4.9
	Sample C	4.8
11	Sample A	4.2
	Sample B	5.0
	Sample C	5.3
	Experimental animals—inulin injected	
Dog		Total inulin chromogen / Inulin space
no.		*mg./100 Gm. white matter* / *Gm./100 Gm. white matter*
8	Sample A	5.0 / 1.2
	Sample B	5.1 / 1.4
10	Sample A	5.8 / 2.0
	Sample B	4.9 / 0.4
	Sample C	5.0 / 0.5
12	Sample A	6.2 / 1.4
	Sample B	8.1 / 3.3
13	Sample A	8.4 / 4.3
	Sample B	5.6 / 1.0
14	Sample A	4.9 / 0.2

TABLE VI

Inulin space of the gray matter in dogs

Dog		Inulinoid substance
	Control animals—no inulin injected	
no.		*mg./100 Gm. gray matter*
7	Sample A	4.6
	Sample B	5.2
	Sample C	4.9
9	Sample A	4.4
	Sample B	4.6
	Sample C	4.7
11	Sample A	4.7
	Sample B	5.3
	Sample C	5.2
	Experimental animals—inulin injected	
Dog		Total inulin chromogen / Inulin space
no.		*mg./100 Gm. gray matter* / *Gm./100 Gm. gray matter*
8	Sample A	5.8 / 3.4
	Sample B	5.4 / 2.1
	Sample C	6.0 / 4.1
10	Sample A	5.1 / 0.5
	Sample B	5.7 / 1.6
	Sample C	5.2 / 0.7
12	Sample A	5.9 / 1.1
	Sample B	5.5 / 0.7
	Sample C	5.8 / 1.0
13	Sample A	9.1 / 5.0
	Sample B	7.6 / 3.3
14	Sample A	6.6 / 1.6
	Sample B	6.3 / 1.4
	Sample C	6.1 / 1.2

Rat nervous tissue

The data obtained for the rats are set out in Table II. The figures obtained apply to the cerebral hemispheres of the rats without reference to

predominance of gray or white matter in the sample taken. The values for the inulin spaces of the tissue were calculated in the same way as in the dogs. The values for the inulinoid content of the cerebral tissues of the control uninjected rats were of the same order as those found in the dogs, expressed per gram of tissue. The inulin space of the rat tissues varied from 1 to 3 per cent of the wet weight of the tissues. These values again are of the same order as those found in the various nervous tissues of the dogs. In the rats, as can be seen from the table, no attempt was made to vary the serum level of inulin from one rat to another, as had been done in the case of the dogs. The experiments in the rats were continued for only three hours, since it has been demonstrated (3, 6, 14) that inulin has diffused fairly completely into the ECF of the rat by the end of three hours. It was felt for this reason that the inulin content of the nervous tissues, after a three hour equilibration period in the rat, should be as satisfactory a measure of the inulin space as in other tissues.

DISCUSSION

These experimental results demonstrate that the blood-CSF barrier, whatever it may be, greatly restricted the passage of inulin from the blood into the CSF of the cisterna cerebello-medullaris. A very significant difference was obvious in the levels of inulin in the blood serum and in the CSF in the ranges studied, although data have not been collected for serum inulin levels above 103 mg. per cent. The results suggest that as the serum level of inulin was raised the amount of it found in the CSF increased slightly, but no consistent relationship was apparent from the limited data available here. Extension of the equilibration time available for the free diffusion of inulin certainly appeared to make little difference in the CSF levels attained.

The small amounts of inulin found in the CSF of the animals in which the highest serum levels had been established may have come across the barrier or may even have diffused through the venous sinuses of the cranium. The relative impermeability of the venous sinus channels and the arachnoid granulations to inulin passing in the reverse direction, that is from the CSF to the blood, has been established by Davson, who injected inulin into the CSF of rabbits and measured its very slow disappearance from the fluid into the blood stream (15).

One disadvantage that is incurred in measuring, as was done here, the inulin content of the fluid withdrawn from the cisterna cerebello-medullaris is that it may not be representative of the fluid as a whole; fluid from the ventricles may contain more inulin which has passed across the choroid plexuses during the experimental period than that fluid already present in the cisterna and available for analysis, although the opposite state of affairs is more likely to be the case. In order to determine how quickly mixing of the ventricular and cisternal fluids may occur, some idea of the rate of formation of ventricular fluid is necessary. The rate of formation and flow studied by the rate of disappearance of substances injected into the CSF suggests that 0.3 per cent of the fluid is renewed per minute by formation in the ventricle (16). More recent studies by Vasilevskii, who determined the volume rate of movement of CSF in dogs by a mathematical analysis of the concentration curves for the transport of an indicator, provide support for this figure (17). According to Vasilevskii (17), 96 to 129 ml. of CSF flows from the ventricles of the dog in 24 hours and the CSF is exchanged not less than 10 times per day. These figures suggest that in the dog at any rate the concentrations of inulin present in the cisternal fluid after an equilibration period of at least three hours are probably not greatly different in order of magnitude from those in the ventricles close to the plexus.

The ratio $\frac{\text{Concentration in CSF}}{\text{Concentration in serum}}$ has been measured for sucrose by Davson, and the value he found was 0.022 (18). In the experiments reported here, a figure of the same order of magnitude was obtained for the ratio in the case of inulin, when allowance has been made for the inulinoid blank values of both serum and CSF. It is difficult to make an accurate comparison of the ratios for sucrose and inulin, since the figure calculated for sucrose by Davson prevailed after the serum level had been maintained at a steady level for a period of time (18), while in the experiments reported here, the serum level of inulin

was presumably at its peak just after injection and slowly declined as the inulin diffused through the ECF of the dog from the time of injection.

From the results of the tissue analysis, it can be seen that just as the blood-CSF barrier restrained the passage of inulin, so the blood-brain barrier also seemed to impair its free diffusion. The low values discovered for the inulin space of the nervous tissue are consistent with the view that the inulin may be confined almost entirely to the blood vascular channels. It has been shown that the average content of blood in the brain of the dog occupies 8.3 per cent of the weight of the tissue (19). Assuming that half of this blood is serum, then 4.1 per cent of the nervous tissue is occupied by serum. This figure is of the same order of magnitude as the values obtained in these experiments for the inulin space of the nervous tissue. The similarity of these two values suggests that inulin diffuses through a space in the nervous tissue closely corresponding in size to that of the blood and most likely confined to the same anatomical location. The low values for the inulin space found in these *in vivo* experiments are in sharp contrast to the values of around 15 per cent of the wet weight of the nervous tissue found by Allen (6). His experiments were carried out on tissue slices of brain and the results can in no way be regarded as inconsistent with ours. They suggest, indeed, that inulin, if it is not imprisoned in the blood vessels by the blood-brain barrier, is free to diffuse more freely through the brain tissue. It is impossible to be sure through what particular fluid phase such diffusion takes place but, considering the extent of the diffusion in Allen's experiments (6), it appears to be similar in extent to what would be regarded as ECF in other tissues. Although Allen found evidence that the inulin entered nerve cells to some extent toward the end of his incubation periods, it is probable that such a finding was more a result of the state of the cells in the tissue slice than a reflection on the behavior of nerve cells *in vivo* with respect to inulin.

The CSF has been described as a transcellular fluid (7, 20) but, as Manery has pointed out (21), it is still part of the body's extracellular fluids and may more specificially be representative in composition of the fluid which bathes the nerve cells, that is, the true ECF of the nervous system or its equivalent. The relative inability of inulin to permeate either the CSF or the tissues of the nervous system, as demonstrated here, may reflect the biological identity of those fluids suggested by Manery (21).

The possibility that the ECF, as it appears to exist in other tissues, is not present in the nervous system has been suggested by Lumsden (22, 23). He contends that the area which makes up the neuropil and lies between the endothelium of the brain capillaries and the nerve cells themselves, consists of a glue-like stuff which is cytoplasm contained in processes of the astrocytes. The nutrients and other substances required by the nerve cells presumably pass from the vascular system across the protoplasmic neuropil either by a process of active transport or by simple diffusion. In the same way the astrocytic processes making up the neuropil may be the system responsible for confining many substances including inulin to the vascular bed of the brain. If this is so then inulin may not permeate the brain fluids because it is unable to pass into and through the cytoplasmic material of the neuropil, just as it does not enter other tissue cells. The means by which this is accomplished is unknown, but it may represent an active transport process on the part of the astrocytes or their processes. The entry of inulin into 14.5 per cent by weight of a tissue slice of brain, as reported by Allen (6), may be regarded in the light of this hypothesis as the result of a failure of the process of active transport under conditions such as exist in a tissue slice, where the oxygen supply of the neuroglia and their processes may be impaired. The results presented here concerning the relative inability of the inulin to penetrate the fluids of the nervous system indicate that the nervous system lacks an inulin space such as exists in other tissues. As it is known that the thiocyanate space in the brain tissue is much smaller than in other tissues (23), the results of these experiments with inulin may be confirmatory evidence of the absence of an ECF phase in the brain comparable to that obtaining in other tissues.

Eichelberger and Richter (24) have analyzed the brain of the dog for chloride and sodium and have calculated its ECF volume, assuming that the

chloride and sodium are extracellular ions. On this basis, they find that the ECF volume of nervous tissue is 30 per cent of the wet weight of the tissue when chloride is the reference ion and 35 per cent when sodium is used as reference. The great discrepancy between the chloride and sodium spaces of nervous tissue as determined by Eichelberger and Richter on the one hand, and the inulin space derived from our experimental results on the other, serves to emphasize the fact that inulin and the other substances used to determine ECF volume in tissues and in the whole organism measure very different things. Quite apart from these considerations, the extreme degree of impermeability of the fluids of the central nervous system to inulin may well be a factor in its small volume of distribution relative to all the other substances used to measure ECF volume in the whole animal.

Inulin is, then, a substance which is probably confined to the blood vascular system in the central nervous system; and the measurement of its content in nervous tissue may well be of use in assessing the degree of damage to the blood-brain barrier. Further study of the relationship of the blood-brain barrier and the ECF of the central nervous system, using methods and substances similar to those used here, may provide further quantitative data on the nature of the fluids of the central nervous system and their relationship to the CSF.

SUMMARY

Inulin was injected intravenously into bilaterally-nephrectomized dogs and rats and, after intervals lasting at least three hours, the cerebrospinal fluid inulin concentration was compared with that of the serum.

Analyses of the gray matter, white matter and spinal cord for inulin were carried out after sacrifice and the inulin space of the tissue estimated.

Under the conditions of the experiment, inulin penetrated the blood-cerebrospinal fluid barrier of the dog to a slight extent. It was not able to cross the blood-brain barrier freely, and the *in vivo* inulin space of nervous tissue was less than 5 per cent of the wet weight of the tissue. The inulin may have been restricted to the blood vascular system either because it was unable to pass across the capillaries of the brain or because a fluid phase corresponding to the extracellular fluid in other tissues does not exist as such in the nervous system.

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TUBULAR SECRETION OF URATE IN MAN *

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In normal man, the renal clearance of urate is only a small fraction, some 5 to 10 per cent, of the inulin clearance. The prevailing interpretation of this low urate/inulin clearance ratio assumes complete filtrability of the plasma urate at the glomerulus, an assumption supported by several lines of evidence (1–7); reabsorption of 90 to 95 per cent of the filtered urate; and excretion of that 5 to 10 per cent which has escaped reabsorption. The renal regulation of urate excretion in man is thus considered to be limited to the processes of glomerular filtration and tubular reabsorption.

There are some difficulties with this concept. Berliner, Hilton, Yü and Kennedy (2), in demonstrating a Tm urate for normal man of the order of 15 mg. per minute, pointed out that this reabsorptive capacity is far greater than any filtered urate load normally imposed upon it. Consequently, the Tm for urate cannot be considered directly to determine the quantity of urate excreted, which is regulated by a proximate mechanism as yet undefined. Not easily reconciled with the current concept is the striking dissociation between filtered urate loads and excreted urate noted over a wide range of glomerular filtration rate in clearance studies of 150 gouty subjects (8). Further, the marked suppression of renal excretion of urate in man after administration of pyrazinamide (9) or lactate (10) would, in the conventional view, require the rather awkward assumption that tubular reabsorption of urate is stimulated by these compounds. Finally, the paradoxical effect of salicylate and other drugs on urate excretion in man (11) would, according to the conventional concept, necessitate the presumption that small doses stimulate, and large doses of the same drug suppress, tubular reabsorption of urate; moreover, that intermediate doses (which have little or no net effect on urate excretion) simultaneously stimulate and suppress the tubular reabsorptive transport system (12).

The present study is concerned with an alternative interpretation of the overall urate clearance data in man which would seem largely to resolve these difficulties, namely that the plasma urate is virtually wholly filtrable at the glomerulus; the filtered urate is then practically completely reabsorbed by the tubules; and tubular secretion of urate constitutes the proximate mechanism largely responsible, under ordinary circumstances, for the excretion of urate in the urine (11, 13). Support for these assumptions is provided by the clearance data to be presented which indicate that, under appropriate experimental conditions, the quantity of uric acid excreted in the urine of man can be demonstrated to exceed the quantity calculated to be filtered at the glomerulus.

METHODS

The subjects studied, seven with intercritical gout and five nongouty, none with overt cardiovascular disease, were given 400 to 600 mg. per day of a potent uricosuric agent, G-28315 (sulfinpyrazone) (14) for a day or two. The clearance studies were then begun, with the patients in the postabsorptive state, by infusing 1 per cent inulin in saline solution and, simultaneously, 0.2 per cent uric acid in 15 per cent mannitol at a rate of 9 ml. per minute (later increased to 17 ml. per minute). Shortly after the infusions were started the first of four doses (each 375 mg.) of the sodium salt of sulfinpyrazone was injected intravenously; this was repeated at intervals of 40 to 60 minutes for a total of 1.5 Gm. to ensure the greatest obtainable suppression of tubular reabsorption of urate throughout the experiment. After allowing for equilibration, urine samples were collected at 15 to 20 minute periods by indwelling catheter, with bladder air wash at the end of each collection period. Venous blood samples were procured at the midpoint of each period, using heparin as anticoagulant.

The uric acid-mannitol infusate was prepared by dissolving 5.0 Gm. uric acid in 1 L. of hot 0.15 per cent lithium carbonate solution; this was then added to 1.5 L. of 25 per cent mannitol in water to give 2.5 L. of 15 per

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cent mannitol containing 0.2 per cent uric acid and 0.06 per cent lithium carbonate. The total uric acid delivered in the course of any one experiment varied from 3.0 to 4.8 Gm. The total quantity of lithium given as Li, 0.2 to 0.3 Gm., is well below the toxic level in patients not receiving salt poor diets (15). No untoward reactions were noted except for transient water emesis in three patients shortly after termination of the experiment. Neither gouty nor nongouty subjects developed any acute arthritis in association with the uric acid infusion.

Inulin was determined in plasma and urine, immediately upon procurement of the specimens, by the resorcinol method of Schreiner (16). Uric acid in plasma and urine was determined by a modification (17) of the colorimetric method of Buchanan, Block and Christman (18) incorporating the use of crude uricase, urea cyanide carbonate, and arsenophosphotungstic acid. As indicated elsewhere (17) the results obtained by this method are in satisfactory agreement with spectrophotometric analysis, which was employed for spot checks.

In making the calculations, the plasma urate was assumed to be completely diffusible in both gouty and nongouty subjects (4). Corrections for plasma protein content or Donnan effect were not made; such corrections, it is estimated, would increase the figures for filtered urate something less than 10 per cent.

RESULTS

Table I summarizes the results of six experiments in which the ratio, excreted urate/filtered urate, exceeded 1.0 in at least two consecutive periods. Ratios > 1.10 were attained in five of these experiments, two reaching 1.20 and 1.23. Of the six similar studies not included in Table I, all yielded ratios of 0.90 to 1.0, and two rose to 1.05 for a single period.

Suppression of tubular reabsorption of urate is required for such demonstration of tubular secretion of urate in man, since the quantity of urate filtered and reabsorbed otherwise is many times greater than that excreted. For this purpose G-28315, which as previously reported (14) markedly increases C_{urate}, was employed. The uricosuric effect was further augmented, about 20 per cent, by vigorous mannitol diuresis to 20 to 40 ml. urine flow per minute, as established in preliminary experiments. Despite these measures, however, it is highly improbable that tubular reabsorption of urate was completely suppressed, even when the excreted urate exceeded the filtered urate. This is unlikely not only on *a priori* but also on experimental grounds; in studies carried out as described under Methods, but omitting uric acid from the infusion, the highest urate/inulin clearance ratio attained was 0.73. It may therefore be presumed that the quantity of urate excreted throughout the experiments, summarized in Table I, was appreciably lessened by persistent tubular reabsorption of substantial amounts of urate.

When the plasma urate was increased by sustained infusion of uric acid to levels of 13 to 22 mg. per 100 ml. (Table I), the filtered urate load of course increased within the limits of the reduced glomerular filtration rates of the subjects studied, but there was a disproportionate increase in excreted urate, the net effect being higher excreted urate/filtered urate ratios. It is presumed that the higher plasma urate levels enhanced the tubular secretion of urate. Moreover, it is likely that the reabsorptive Tm for urate, which was markedly reduced by the G-28315 administered, was exceeded in some experiments by the large filtered urate loads presented to the tubules.

A prerequisite for the demonstration of urate/inulin clearance ratios > 1.0 in these studies was a reduced glomerular filtration rate, of the order of 40 to 80 ml. per minute. This requirement was fulfilled by selection of subjects known through prior clearance studies or suspected by virtue of age to qualify. As indicated in Table I, the experimental conditions employed did not effect any significant change in inulin clearance. It was not necessary to resort to positional changes or use of ganglionic blocking agents to bring about any further reduction in the glomerular filtration rate.

DISCUSSION

The data summarized in Table I offer direct testimony for tubular secretion of urate in normal and gouty man. It should be emphasized that the excreted urate/filtered urate ratios cited were procured in the face of probable incomplete suppression of tubular reabsorption of urate, and therefore should be regarded as minimal figures. Although quantitative data thus are not available, it may be inferred that under ordinary circumstances the excreted urate is very largely derived by tubular secretion (8). Indeed it is possible that larger quantities of urate are secreted by the tubules than are excreted in the urine, since the sites of secretion and reabsorption of urate in the

TABLE I

Experiments demonstrating tubular secretion of urate in gouty and nongouty subjects

Name Age B.S.A.	Time	Urine flow	C_{inulin}*	P_{urate}†	F_{urate}‡	UV_{urate}§	C_{urate}‖	Excreted urate / Filtered urate
	min.	*ml./min.*	*ml./min.*	*mg./100 ml.*	*mg./min.*	*mg./min.*	*ml./min.*	
1. M. H.	–42	Infusions started: inulin; uric acid-mannitol, 9 ml./min.; G-28315¶						
49	0–99	11.1	68.9	14.0	9.65	7.53	53.7	0.78
1.85	99–134	16.9	75.7	16.5	12.5	9.79	59.3	0.78
Gout	134	Uric acid-mannitol infusion rate increased to 17 ml./min.						
	134–149	21.3	68.8	18.8	13.0	12.2	65.0	0.94
	149–159	24.5	64.5	19.2	12.4	14.0	73.0	1.13
	159–174	28.3	67.0	20.4	13.7	15.8	77.3	1.15
	174–184	30.0	66.3	22.0	14.6	17.4	79.3	1.19
	184–189	30.0	66.3	22.3	14.8	17.4	78.0	1.18
2. I. K.	–42	Infusions started: inulin; uric acid-mannitol, 9 ml./min.; G-28315¶						
65	0–61	13.2	44.3	10.2	4.52	3.67	36.0	0.81
1.73	61	Uric acid-mannitol infusion rate increased to 17 ml./min.						
Gout	61–94	21.1	43.0	13.6	5.85	6.22	45.7	1.06
	94–107	23.5	40.5	15.7	6.35	7.20	46.0	1.13
	107–121	25.4	39.3	17.0	6.70	8.20	48.3	1.23
	121–138	28.0	46.4	18.6	8.60	9.40	50.5	1.10
	138–146	29.7	48.0	18.6	8.90	10.0	53.8	1.12
3. H. G.	–32	Infusions started: inulin; uric acid-mannitol, 9 ml./min.; G-28315¶						
68	0–63	20.5	54.0	8.5	4.60	2.09	24.3	0.46
1.51	63–103	22.5	58.7	13.8	8.10	4.78	34.7	0.59
Gout	103–123	23.1	62.0	13.0	8.06	6.65	48.2	0.83
	123–143	23.9	60.2	13.4	8.05	8.01	58.0	0.99
	143–162	27.5	60.3	13.6	8.20	9.15	67.3	1.12
	162–173	28.6	62.2	13.8	8.60	9.68	70.3	1.13
4. J. R.	–38	Infusions started: inulin; uric acid-mannitol, 9 ml./min.; G-28315¶						
47	0–98	8.6	86.4	11.1	9.60	5.98	53.8	0.62
1.83	98–160	13.0	82.7	13.6	11.3	8.82	64.9	0.78
Gout	160	Uric acid-mannitol infusion rate increased to 17 ml./min.						
	160–180	17.8	69.0	15.1	10.4	11.3	74.8	1.09
	180–195	18.3	69.5	15.8	11.0	11.4	72.2	1.04
	195–207	21.3	72.8	16.3	11.9	12.4	76.0	1.04
5. H. M.	–51	Infusions started: inulin; uric acid-mannitol, 9 ml./min.; G-28315¶						
65	0–59	17.7	81.4	8.0	6.50	5.48	68.5	0.84
1.74	59	Uric acid-mannitol infusion rate increased to 17 ml./min.						
Nongouty	59–74	22.3	86.0	10.0	8.60	8.04	80.4	0.93
	74–89	29.3	81.3	11.7	9.50	10.6	90.7	1.12
	89–104	33.3	84.2	12.7	10.7	12.0	94.5	1.12
	104–119	37.3	87.0	13.9	12.1	13.5	97.2	1.11
	119–134	42.7	85.0	15.2	12.9	15.5	102	1.20
6. L. W.	–45	Infusions started: inulin; uric acid-mannitol, 9 ml./min.; G-28315¶						
72	0–57	8.5	49.2	11.3	5.55	4.72	41.8	0.85
1.60	57	Uric acid-mannitol infusion rate increased to 17 ml./min.						
Nongouty	57–72	13.0	53.0	12.9	6.85	6.15	47.7	0.90
	72–84	16.3	48.3	13.7	6.60	7.59	55.3	1.15
	84–94	18.0	51.7	14.7	7.60	8.19	55.8	1.08
	94–100	20.8	52.0	15.7	8.15	9.03	57.5	1.11

* Inulin clearance.
† Plasma urate concentration.
‡ Filtered urate.
§ Urinary urate excretion.
‖ Urate clearance.
¶ Injection of G-28315 repeated every 40 to 60 minutes three times during the course of the experiment.

tubules have not been established, and reabsorption of some secreted as well as filtered urate is not precluded by the overall clearance data. In special circumstances (inborn or acquired defects in tubular reabsorption of urate, use of uricosuric drugs) a substantial proportion of the excreted urate would include filtered urate which has escaped reabsorption.

Praetorius and Kirk (19) previously demonstrated tubular secretion of urate in a young man with striking hypouricemia but in good health. This represents a special case, however, since the clearance data disclosed a marked intrinsic deficiency in tubular reabsorption of urate, the human equivalent of the Dalmatian coach hound.

In the present study, tubular secretion of urate could be as readily demonstrated in gouty as in nongouty subjects, and no gross differences were disclosed. Although these data do not, of course, exclude the possibility of deficient tubular secretion of urate in gout, this is considered unlikely for reasons discussed elsewhere (8). The data do make clear, however, that the tubular reabsorption of urate in gouty or nongouty subjects cannot be estimated indirectly by clearance calculations which assume no tubular secretion of urate, hence comparisons on this basis are invalid (8).

SUMMARY

Excreted urate/filtered urate ratios > 1.0 were demonstrated by appropriate clearance techniques in gouty and nongouty man, implying tubular secretion of urate. The results support the hypothesis that the renal regulation of urate excretion in man involves glomerular filtration, tubular reabsorption and tubular secretion.

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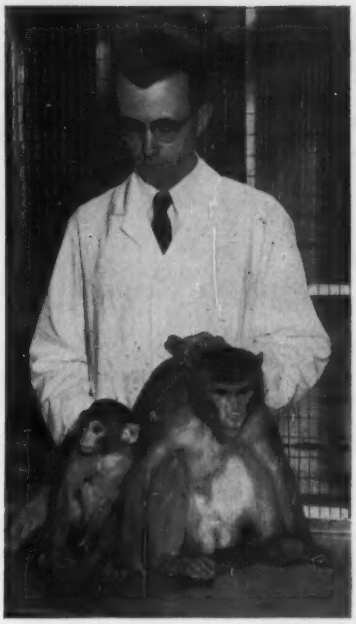

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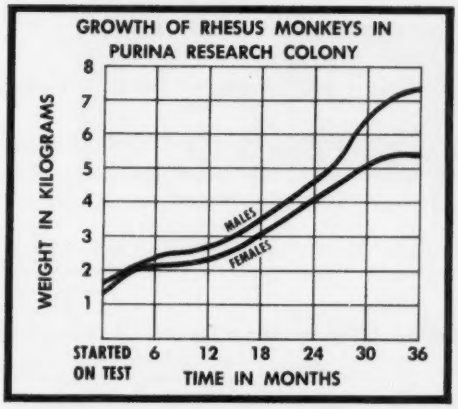

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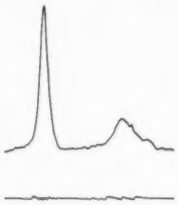

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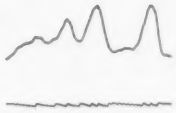

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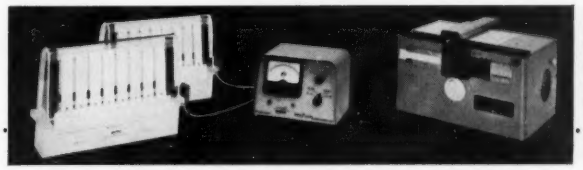

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TWO PARTS
PART II

The Journal of Clinical Investigation

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

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INTRODUCTION

The year 1959 marks the fiftieth anniversary of the American Society for Clinical Investigation and the thirty-fifth year of its publication, the Journal of Clinical Investigation. It appeared fitting to the Council of the Society that the history of the Society and the Journal be recorded on the occasion of these anniversaries.

The further decision to publish these histories as a supplement to the Journal of Clinical Investigation requires some justification. The vicissitudes of an organization may be of interest to its members; but should nonmembers be expected to evince a comparable interest? Is it justifiable to publish a lengthy historical dissertation in a journal devoted entirely to scientific material? We feel that these questions should be answered in the affirmative. The readers of this Journal are not total strangers. They constitute a small and select group of scientists whose interest in clinical investigation binds them into a community which has a strong common interest with the Society and the Journal. Whether or not they are actual members of the organization, they participate in activities which the Journal and the Society were organized to encourage. During the years since their founding the Society and its publication have both molded and reflected the evolution of clinical investigation. The activities of all of our readers have been affected by the interests and influence of the Society. It therefore appeared probable that most clinical investigators would be interested in the perspective offered by a review of the early days of clinical investigation and by the part played by these organizations in the development of the present pattern of medical research. With these considerations in mind, the Council of the Society decided to underwrite the publication of these histories in the Journal of Clinical Investigation.

The compilation of this supplement was time consuming and difficult. It had originally been hoped that one of the distinguished emeritus members of the Society would undertake to write its history, but the task proved too demanding and the several candidates gracefully declined. Fortunately, a member of the editorial secretarial staff of the Journal who had been trained as a writer and historian was willing to undertake the burden. Mrs. Ellen R. Brainard has therefore written the histories. She was greatly aided by many of our members and emeritus members. We are grateful to them for their help. In addition to those whose communications are acknowledged in the text, we are particularly indebted to Drs. Dana Atchley, C. Sidney Burwell, Allan M. Butler, Joseph M. Hayman, Elliott P. Joslin, Roger I. Lee, Robert F. Loeb, Thomas M. Rivers and W. Barry Wood, Jr., for personal reminiscences and unpublished material, and to Drs. Arthur Bloomfield, Saul J. Farber, John A. Luetscher, Jr., J. Howard Means, and John R. Paul, who reviewed the manuscripts and made many helpful suggestions.

—PHILIP K. BONDY

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The editors of the *Journal of the American Medical Association* for permission to reprint, in its entirety, the presidential address of Dr. S. J. Meltzer, "The Science of Clinical Medicine: What It Ought To Be and the Men To Uphold It" (J. Amer. med. Ass. 1909, **53**, 508–512).

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HISTORY OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION, 1909–1959

By ELLEN R. BRAINARD

I. ORIGINS

Beginnings on the Boardwalk

Although the age of the American Society for Clinical Investigation is reckoned formally from the date of its first annual meeting—May 10, 1909—its story really begins two years earlier, in June, 1907, when the idea for its formation was first suggested.

The man behind the idea was Dr. Samuel J. Meltzer; the setting, the Boardwalk in Atlantic City, New Jersey. The story, recounted for Society members by Dr. Warfield Longcope (1) is as follows:

> A few of you will remember the circumstances under which this Society originated and the modest manner in which it was launched for action. One afternoon, after a session of the Association of American Physicians[1] here in Atlantic City, a little group of men stopped in their promenade on the Boardwalk to talk. Dr. Meltzer happened to join them and soon led the conversation to the topic of medical meetings. In his cheerful and enthusiastic manner which we all know so well, he suggested that a new society should be formed with a somewhat different aim and a different membership from those already in existence. He felt very strongly that there should be an opportunity for the men, most of who were young, and actively engaged in clinical investigation, to come together and discuss their work. Indeed, a requisite for admission to the Society was that a man should be actively engaged in investigation and a clinician. Thus this society, he hoped, would stand for investigation in internal medicine, as distinguished from those societies which represented investigation in the fundamental sciences.

As nearly as we can discover, the group on the Boardwalk included, in addition to Drs. Longcope and Meltzer, Dr. David L. Edsall, Dr. Wilder Tileston and probably Dr. Joseph H. Pratt. Their recollections substantiate and augment Dr. Longcope's story and help us to imagine what the gist of their conversation must have been.

"The reason for the starting of the Society," according to Dr. Tileston (2), "was that at the meetings of the Association of American Physicians (whose membership was limited at that time to 160), the younger men had few opportunities to present their papers, since many of them were not members."

Further illumination is provided by Dr. Edsall (3):

> I have a very sketchy memory of what happened in any detail at the time of the formation of the American Society for Clinical Investigation. I remember chiefly meeting Dr. Meltzer on the board walk at Atlantic City when the matter first came up and his making the suggestion . . . of the formation of the Society in order to give younger men who were not yet members of, and not eligible for, the Association of Physicians the advantages of meeting other young men active in work of that kind and discussing matters with them, and the stimulus that would come from that.

Dr. Meltzer's suggestion apparently met with a ready response. That same evening he invited the group to his hotel room, most likely at the Traymore, to explore the idea more fully. Drs. Henry A. Christian and Rufus Cole may have been included at this meeting and, although there is no formal record of what went on, it is clear that the idea took hold. The outcome was the formation of a committee consisting of Drs. Christian, Edsall, Pratt and Tileston, which met in Boston on June 17, 1907, at the University Club.

At this meeting, the four men discussed the aims and scope of the proposed society and agreed to enlarge the committee by the inclusion of Drs. Cole, Longcope and Meltzer. Before the next meeting, Dr. J. A. Capps of Chicago and Dr. A. W. Hewlett, then of San Francisco, were added as well, bringing the total number on the founding committee to nine and extending its geographical distribution westward (the other seven were all from New York, Philadelphia, Baltimore or Bos-

[1] Actually, it was a session of the American Medical Association rather than of the Association of American Physicians, although the latter was an understandable error made in retrospect by several of the early members. The Society's minutes state that informal discussions were held at the time of the American Medical Association meeting in 1907. Furthermore, all personal accounts agree that these discussions took place in June, 1907, in Atlantic City. The Association of American Physicians had met in May of that year in Washington, whereas the American Medical Association convened in Atlantic City June 4–7, 1907.

ton). Sometime, probably in the interim between this first meeting and the second, which took place that autumn, a list of men considered eligible for membership was drawn up and divided among the committee members. Each was responsible for checking the publications record of a group of nominees for the previous five years, this record to be used as a basis for selection. It was agreed that seven of the nine must approve each nominee before an invitation to share in the society's organization would be extended to him.

The committe, with the exception of Dr. Hewlett, met again on November 7, 1907, at the Manhattan Hotel in New York at the time of a meeting of the Interurban Clinical Club. It is probably this meeting of which Dr. Longcope (4) wrote:

> I remember . . . that we all sat about a table somewhere and discussed at considerable length a list of names from which the membership of this new society should be recruited. As I recall the discussion, these men were selected because it was thought they would be interested in the new endeavor and were sympathetic with the idea of forming a society whose main object was to discuss the problems in clinical investigation in which each and every one was engaged.

The result of these deliberations was a list of 22 men to whom an invitation to be present at the organizational meeting was sent.[2] A committee of three—Drs. Christian, Longcope and Meltzer—was appointed to draft a constitution to be presented at the charter meeting, set for the day prior to the 1908 meeting of the Association of American Physicians in Washington. It is likely, too, that the matter of a name for the nascent society was discussed at the November committee meeting. With regard to the somewhat cumbersome choice —"American Society for the Advancement of Clinical Investigation"—Dr. G. Canby Robinson offers the following explanation:

> As I recall, the word "Investigation" was used instead of "Research" because there was another society composed of homeopaths known as the Society of Clinical Research (5).

[2] The list is included below in the account of the charter meeting. It was either expanded by the addition of three names prior to the organizational meeting or the list given in the minutes of the November committee meeting is incomplete, for the records of the charter meeting (May 11, 1908) contain the names of 25 men (in addition to the nine committee members) who had been invited to be present.

Thus, by November—a scant four months after Dr. Meltzer had first proposed it—the idea had been given concrete form and the new society was almost a reality. It was formally constituted on May 11, 1908, at the New Willard Hotel in Washington.

The charter meeting convened at 3:30 p.m. with 17 men present for Dr. Edsall's call to order. Edsall was elected temporary chairman; Dr. Christian, temporary secretary. Consideration of the constitution was the first item on the agenda and it was agreed to read, discuss and vote on it article by article. Most of the amendments to the original draft involved only minor modifications in wording. The minutes hint, however, at what must have been a lively discussion regarding a point in Article III, Section 1—"Eligibility for Membership"—which read: "Any practicing physician residing in the United States or Canada who has accomplished a meritorious, original investigation in the clinical or allied sciences of medicine . . . is eligible to membership." Dr. T. C. Janeway's inquiry as to what was to be the criterion for judging whether a man was a "practicing physician" set off a discussion in which several of the members participated. Would a resident at the Johns Hopkins Hospital, for example, be considered a practicing physician, Dr. Janeway wanted to know. Unfortunately, the minutes do not elaborate the points raised in reply and, although it must have been an interesting debate, we do not know whether Dr. Janeway received a satisfactory answer to his question. In any case, no alteration in the section resulted.

One change, which may have been significant, involved Article VII—"Meetings"—which read, originally, "The Society shall hold one general meeting annually. This meeting shall take place in the spring on the date preceding and at the place of the annual meeting of the Association of American Physicians." On Dr. Cole's motion it was voted to delete all but the first sentence, thereby leaving the time and place open to the discretion of the Council. Whether or not Dr. Cole's amendment reflected reluctance to fashion the new society so obviously in the footsteps of the old is, of course, not on record and, amendment or no amendment, the Society, in fact, met regularly in geographical and temporal

proximity to the Association. The remaining articles were approved with little debate, and the constitution as a whole was formally adopted by the Society.

Following this action, the chair was empowered to appoint a three-man nominating committee which presented a single slate of candidates for office. Until the fourth annual meeting, apparently each member of the Society cast an individual ballot for the slate. This formality was abandoned in 1912 when it was moved that the secretary cast a unanimous ballot for all nominees, a procedure which has been followed ever since, though not without occasional criticism. The officers elected were as follows: Dr. Meltzer, president; Dr. Edsall, vice-president; Dr. Christian, secretary; Dr. John Howland, treasurer; and Drs. Miller, Janeway and Hewlett, councillors for one, two and three years, respectively.

Dr. Meltzer took the chair, and the remainder of the meeting was devoted to approval of the order of business for the first annual meeting, appointment of a committee to draft by-laws to the constitution, approval of a Council recommendation that dues for the forthcoming year be set at $5.00, and the nomination of 20 men for membership. It is interesting to note that for the first few years all nominations were made at the meetings and were voted upon the following year. The minutes carefully record, along with the name of each nominee, the name of the members who proposed and seconded his nomination. The nominating procedure was altered by constitutional amendment in 1913; thereafter, nominations could be made in writing at any time during the year.

Letters of invitation had been sent to 34 men, including the nine committee members. Of these, one had apparently declined; 30 had accepted; and three, two of them in Manila, had not been heard from at the time of the meeting. (Replies indicating acceptance were eventually received from the two gentlemen in the Philippines; the third apparently never answered or declined the invitation to join.) The final count of 32 physicians who indicated their desire to participate in the new society is presented in Table I. It was moved that these men be considered charter members, with the proviso that in order to be so considered, members who had not attended the organization meeting would be present for the first annual session the following year. Those who attended one or both of the two meetings, as noted in the minutes, are indicated in the table. Presumably it is only these who are officially considered charter members, although various lists contain discrepancies in this regard.

TABLE I

Physicians accepting invitation to join the American Society for Clinical Investigation, 1908

B. K. Ashford	United States Army
E. R. Baldwin*	Saranac Lake, N. Y.
W. J. Calvert*	Columbia, Mo.
J. A. Capps*	Chicago, Ill.
H. A. Christian*†	Boston, Mass.
R. Cole*†	Baltimore, Md.
D. L. Edsall*†	Philadelphia, Pa.
D. M. Cowie	Ann Arbor, Mich.
C. P. Emerson	Baltimore, Md.
H. Emerson†	New York, N. Y.
M. Fischer*	San Francisco, Cal.
N. B. Foster†	New York, N. Y.
A. W. Hewlett*	Ann Arbor, Mich.
J. Howland*†	New York, N. Y.
J. R. Hunt†	New York, N. Y.
T. C. Janeway*†	New York, N. Y.
E. Libman*†	New York, N. Y.
E. A. Locke*†	Boston, Mass.
W. T. Longcope*†	Philadelphia, Pa.
D. J. McCarthy	Philadelphia, Pa.
S. J. Meltzer*†	New York, N. Y.
J. L. Miller*	Chicago, Ill.
W. E. Musgrave	Manila, P. I.
J. H. Pratt†	Boston, Mass.
M. Richardson*	Boston, Mass.
E. C. Rosenow	Chicago, Ill.
J. Sailer*†	Philadelphia, Pa.
C. E. Simon	Baltimore, Md.
J. D. Steele‡	Philadelphia, Pa.
R. P. Strong	Manila, P. I.
W. Tileston*†	Boston, Mass.
G. B. Wallace	New York, N. Y.

* Present at 1908 charter meeting.
† Present at first annual meeting, 1909.
‡ Dr. Steele died in 1908.

With the adjournment of the meeting that evening, the American Society for the Advancement of Clinical Investigation was formally launched. Before continuing the story of its subsequent growth, it might be well to examine more fully the background of its origins, for the founding of the Society was closely related to the broader picture of contemporaneous developments in American medical research. In a very real sense, the Society grew up with investigative medicine in this country, and the "rebellion" of the Young Turks was a part of the spirit of the times. In

an attempt to understand that spirit, we shall look more closely at the men who laid the plans for the new organization and at both the immediate considerations and the far-reaching ideas which motivated them.

Young Turkmanship: Nine Men and Their Motives

Back in the days when the term could have been used with dignity and meaning, **Dr. Meltzer** was a true "organization man," one who was firmly convinced of the value of learned societies for the stimulation of creative thought and activity. In 1903, he had been instrumental in the founding of the Society for Experimental Biology and Medicine, popularly called the "Meltzer Verein"; in 1905, his enthusiastic support had urged the Harvey Society into existence (6). He is reported (7) to have overwhelmed the objections that such a society would never succeed in a city as lacking in scientific interests as New York by saying to the group of men assembled to discuss its possibility: "Never mind if no one comes except ourselves. We will wear our dress clothes, sit in the front row, and show the speaker that we appreciate him."

It must have been with the same ardent conviction that Dr. Meltzer inspired his companions on the Boardwalk with the idea of forming still another society, yet one which was not to be *just* another society. His views as to what it should represent were very positive ones and provided the foundations on which the young organization was built. Yet it is curious that Meltzer himself was not a member of the original four-man committee which took the first steps in transforming idea into action. In seeking the reason, another facet of his personality comes to light. Dr. Tileston (2) offers the following explanation:

> Dr. Meltzer from the beginning decided to take no part in the organization of the Society but to leave that to the younger men. He was a modest man, and much older than the others, and he preferred to stay in the background. That accounts for the fact that he was not a member of the committee that met in Boston in 1907, and did not attend that meeting.

Dr. Meltzer was, in 1907, 56 years old and, in the words of Dr. Pratt (8), "took a fatherly interest in the young clinicians"—Longcope, then 30 years of age; Christian, 31; Tileston, 32; Hewlett, 33; Capps, Cole and Pratt, 35; and Edsall, 38. These young men were a diversified group in terms of interests, educational background, and the subsequent patterns of their professional careers. But all were representative of the type of man on whose shoulders Dr. Meltzer, addressing them as the Society's first president, was to place the responsibility for upholding "the science of clinical investigation." The concept of such a science was a radical one at the turn of the century. It was to become commonly accepted during the lifetime of the younger men. Changes beginning to be felt about 1900 were to make of medicine a strikingly different discipline from that which men of Dr. Meltzer's generation had known. There could be no more fitting illustration of some aspects of this changing picture than the professional career of Dr. Meltzer himself.

Samuel J. Meltzer. Born in Courland, Russia, in 1851; went, at an early age to Konigsberg and thence to Berlin to study medicine under "the best faculty in Europe"—Virchow, Leyden, Frerichs and Kronecker; turned down a position at the University of Berlin to come to the United States; made several trans-Atlantic trips as a ship's doctor until, in 1883, he had acquired the means to establish an office of his own in Harlem; built a large and lucrative practice; carried out physiological and pharmacological experiments in whatever spare hours he could find; in 1904, closed his offices to become head of the Department of Physiology and Pharmacology at the newly-established Rockefeller Institute for Medical Research; there, until his death in 1921, devoted himself to studies of major importance to medicine—the role of magnesium in the organism in health and disease; inhibition as a positive factor as opposed to excitability; anesthesia, especially intratrachial insufflation; the effects of epinephrine (9).

Dr. Meltzer was an extraordinary man: an avid reader of several languages; possessor of an extremely retentive memory; above all, wholly dedicated to the science of medicine.

Of his student days in Germany, we have the following rather amusing anecdote:

> While working in Kronecker's laboratory, he (Meltzer) studied the mechanism for swallowing, which involved passing into his own stomach a double barrelled stomach tube. At this time, a bill was pending in the

Reichstag for the abolition of animal experimentation. It claimed that doctors carried out on animals what they feared to do on themselves. The Minister of Health, while himself making a tour of inspection, entered the laboratory while Meltzer was recording notes with this ungainly tube in his stomach. The inspector requested its removal, asked about Meltzer's studies, and then used this episode for the defeat of the impending bill. He requested all members of the SPCA to submit themselves for study to Dr. Meltzer. None accepted his challenge (9).

After his sojourn in the German university clinics, Dr. Meltzer must have found the scientific atmosphere—or rather, the lack of it—in America rather stifling. During the post-Civil War decades, research—medical and otherwise—received virtually no support from either government or commercial sources and very little from private subsidy. The scientific studies of the French and German clinicians had made a slight impression on the native medical outlook, at least among the upper echelons of the profession, but medicine remained, by and large, a practical art based on "tried and true" remedies. There were only three or four medical centers in the country where the sciences were systematically pursued. Isolated individuals with pitifully inadequate equipment carried out such experiments as they could find time for in the daily routine of practice. Dr. Meltzer's experience was typical:

After visiting his patients, he (Meltzer) would drive to the physiological laboratory of the College of Physicians and Surgeons, tie his horse to a lamp post, and perform some physiological experiment. Many experiments were also carried out in his own little house, often late at night (6).

During the closing years of the century, however, a brighter future seemed imminent. The activities of men engaged in research, both here and abroad, began to emerge from the phase of mere classification and description of disease and to show promise of results which would contribute to progress in the prevention and cure of human illness—a development which was of particular importance if scientific medicine were to appeal to the practical-minded American public. At the same time, the period following the Civil War had been characterized by spectacular accumulation and concentration of wealth and individual fortunes had risen to previously undreamed of heights. The possessors of such wealth, who previously had been inclined to patronize religion or the arts, began to see in science something worthy of a second thought.

In 1867, Johns Hopkins, a Baltimore merchant and financier, decided to use his sizable fortune for the endowment of a university and hospital and, when he died in 1873, he left $7 million to be divided equally between the two (10). This was the largest individual gift bestowed upon a public institution up to that time. It was Hopkins' express command that the hospital should be of such high standards as to form, eventually, an integral part of the university medical school, a goal which was visionary indeed for a period in which most hospitals were conducted by local philanthropies or religious groups and most medical schools were in reality private businesses lacking anything resembling university standards. The hospital was opened in 1889; the medical school, in 1893. Hopkins' ideals were guided into reality by Daniel C. Gilman, first president of the university, and John Shaw Billings who planned and supervised the construction of the hospital and formulated principles for the development of a medical school which, with William Henry Welch as its first dean, was to become the exemplar for the vast improvements in American medical education which took place during the early decades of the twentieth century.

After Hopkins, the principle of private subsidy for science began to gain favor. The story of the founding of the Rockefeller Institute is no doubt a familiar one. In 1901, the Board received from the Rockefellers $20,000 to be distributed as grants for medical research (11). The concept of providing funds to assist individuals during the course of investigations was, at that time, an innovation in striking contrast to the more usual practice of rewarding researchers, if at all, at the termination of their work. By using private wealth to stimulate the initiation of scientific studies, the Rockefeller grants set a precedent later followed by other philanthropists, the American Medical Association, private research councils and the federal government. The grants also served to gauge the country's readiness for the establishment of a permanent institute for research. The response indicated the existence of sufficient interest and personnel for such a venture, and in 1903 the grant program was terminated and a permanently endowed research laboratory built in New York.

The following year, Dr. Meltzer, then 53 years old, joined the Institute's staff and embarked on the final stage of his career. It was a turning point for American medical research as well for, while it is unlikely that all investigators suddenly found themselves in possession of well-equipped laboratories and unlimited funds, it is certainly true that up-and-coming young men were no longer beset by the kinds of difficulties which had thwarted the research ambitions of men of Dr. Meltzer's generation. To them, instead, fell the tremendous challenge of putting their expanding opportunities to good use. This was to be no easy undertaking. It involved not only delving into the fundamental mysteries of health and disease, but attacking such corollary problems as the conversion of the medical profession to the view that these mysteries needed to be explored and could best be solved by the use of scientific techniques. Mobilization of the profession required, in turn, a drastic upgrading of the standards and methods of medical education. Among the men, fittingly designated "Young Turks," who dedicated themselves to these tasks there were:[3]

Joseph Almarin Capps (1872–) who was born in Illinois and, after coming East for his medical training (M.D., Harvard, 1895) and internship (in several Boston hospitals), returned to his native Midwest in 1904 to become Assistant Professor of Medicine at Chicago's Rush Medical College. Between 1919 and 1926, he was Professor of Medicine there and, in 1926, assumed the position of Professor of Clinical Medicine at the University of Chicago. He was also Chief of Medical Services at Cook County Hospital. His principal scientific studies dealt with morphology of the blood, respiratory infections such as epidemic streptococcosis, physiology of the pleura, pain sense in the peritoneum and pericardium, and venous blood pressure.

Henry Asbury Christian (1876–1951), a Virginian who came North after receiving the M.D. degree from Johns Hopkins University in 1900 and soon thereafter began to make medical history in the city of Boston. His first positions were those of Assistant Pathologist at the Boston City Hospital and Instructor in Pathology at Harvard Medical School. In 1906, by this time Instructor in the Theory and Practice of Physic, he established a laboratory of experimental medicine at Harvard. In 1908, at the age of 32, he became Hersey Professor of the Theory and Practice of Physic (a position he held until 1939) as well as "the boy dean" of the Harvard Medical School (1908–1912) which was at that time undergoing revitalization at the hands of University President Charles W. Eliot. He and his colleague, Dr. Edsall, fathered the "Harvard full-time system" in clinical teaching (13). In 1910, Dr. Christian was appointed Physician-in-Chief of the new Peter Bent Brigham Hospital (opened in 1913) and was largely responsible for organizing and developing it as an outstanding teaching hospital of Harvard University (10). There he instituted a system of student clinical clerkships (Dr. Edsall was simultaneously doing the same at the Massachusetts General Hospital), a system which had been imported from Great Britain and put into effect under Dr. William Osler at Johns Hopkins, but which was otherwise an innovation in American medicine (13). The system was designed to give fourth-year medical students real responsibilities in the care of patients as members of a hospital ward team and, as such, it added a significant dimension to their medical training. At the Peter Bent Brigham Hospital, in 1913, Dr. Christian introduced the first electrocardiograph ever to be used in a general hospital in this country. He retired from active service as Physician-in-Chief in 1939. Three years later, he took on new duties as Clinical Professor of Medicine at Tufts Medical School and Visiting Physician to the Beth Israel Hospital of Boston.

Dr. Christian's published studies, on pathological and clinical subjects, dealt primarily with cardiovascular diseases, nephritis, diuretics and multiple myeloma.

Rufus Cole (1872–) received the B.S. degree from the University of Michigan in 1896 and began his medical studies there. During one of his summer vacations, however, he visited the Chicago World's Fair and saw there an exhibit of the newly organized Johns Hopkins Medical School (14). Deeply impressed, particularly by

[3] In addition to references not specifically cited in the text, the following sources have been used, particularly for information regarding dates and professional positions, in compiling these biographies: History of the Interurban Clinical Club, 1905–1937 (12) and appropriate editions of Who's Who in America and American Men of Science.

the plans for the anatomical laboratory being developed under Dr. Mall, he arranged to transfer to Hopkins for his final year of medical studies, a feat of considerable difficulty since the faculty of medicine had shown great reluctance to admit students who had not, from the beginning, been imbued with the approach to the study of medicine then being developed in Baltimore. Following his graduation in 1899, he held, until 1909, positions at Hopkins ranging from Resident to Associate in Medicine. During these years he participated in important phases of Hopkins' transition into a research-centered institution comparable to the great German universities. He was put in charge of the biological research laboratory established in Dr. Lewellys F. Barker's clinic and there engaged in Hopkins' first systematic clinical research (10).

In 1908, Dr. Cole was offered the first directorship of the new Hospital of the Rockefeller Institute for Medical Research, "new" not only in terms of facilities but in terms of the idea for which it stood.[4] After accepting the position, he spent several months abroad to acquaint himself with research activities in Europe, particularly those of the German university medical clinics. Although these were far in advance of anything in America, none completely embodied the vision which Dr. Cole and the Rockefeller Institute's Board of Scientific Directors hoped to realize in the Hospital (10). Thus, in formulating the plans for its construction and organization, Dr. Cole worked largely without precedent. He devoted nearly 30 years, until his retirement in 1938, to the development of this Hospital which itself became the prototype for medical research institutions all over the world. During these years, Dr. Cole continued his own scientific studies. His major interest was the field of infectious diseases and immunity, and his contributions, particularly with respect to lobar pneumonia, were outstanding ones.

David Linn Edsall (1869–1945) was among the young physicians who, about the turn of the century, experienced first-hand the contrast between the European and American medical research climates and who, upon returning from their studies abroad, began to do something about the medical backwardness of their own country. Edsall was graduated from the University of Pennsylvania with the degree of M.D. in 1893 and, following his internship, pursued his studies (in 1894–95 and in 1901) in London, Vienna, and Graz. Between 1895 and 1910, he held various positions at the University of Pennsylvania including those of Associate at the William Pepper Laboratory, Assistant Professor in Medicine, and Professor of Pharmacology and Therapeutics. During this period, he developed an interest in the medical problems of industry, contributing studies on the prevention of industrial disorders which evidenced his awareness of environment as an important factor in determining the complete clinical picture (15). This early interest in social aspects of medicine was carried over later in his career when in 1922 he became Dean of the Faculty of the Harvard School of Public Health.

His contributions to medical education were of particular significance. Although he spent only a single year at Washington University in St. Louis (as Professor of Preventive Medicine in 1911–12), many of his ideas were influential in the reorganization of that university's medical school which was taking place at that time (10). In 1912, he was appointed Jackson Professor of Medicine at Harvard and Chief of Medical Services at the Massachusetts General Hospital (the first non-Bostonian, incidentally, ever to hold these positions simultaneously). Thereafter, he worked with Dr. Christian in bringing the full-time system into being at Harvard, first, by picking a group of interns from Massachusetts General Hospital and preparing them for his full-time staff by sending them abroad or to research laboratories in this country for experience in the methods of investigation; then, by transforming administrative offices into research laboratories, raising funds for equipment and salaries and, finally, putting his teaching staff to work on various lines of clinical investigation (13).

In 1918, he was appointed Dean of the Harvard Faculty of Medicine (until 1923, he held this position simultaneously with his professorship) and, in 1922, Dean of the Faculty of the School of Public Health, posts at which he remained until his retirement in 1935. His own major field of investigation was concerned with metabolic disturbances.

[4] Dr. Cole's work both at Johns Hopkins and at the Rockefeller Hospital is discussed in greater detail below.

Albion Walter Hewlett (1874–1925) was a native Californian who came East as a member of the fourth class to enter the Johns Hopkins Medical School from which he was graduated with the M.D. degree in 1900. He returned to San Francisco for two years as Assistant Professor of Medicine at Cooper Medical College (1906–08), then became Professor of Internal Medicine at the University of Michigan (1908–16) and returned once again to the West Coast to become Professor of Medicine at Stanford University until his death in 1925. His scientific studies dealt with irregularities of the heart, the blood flow in the arm, and other subjects in the field of pathological physiology.

Warfield Theobald Longcope (1877–1953) exemplified the type of teacher of medicine who was to come into increasing prominence during the first decades of the Society's existence—the teacher whose primary interest was research rather than practice. Except for part of one year when he entered practice in New York City, Dr. Longcope devoted himself to teaching and investigation and limited his direct contact with patients to that connected with his responsibilities in university-affiliated clinics (10). He was a modest, unassuming man who, in his teaching, took a deep interest in his junior associates and communicated to many of them not only his great wealth of knowledge about disease but his enthusiasm for the study of medicine (16).

Longcope was graduated from Johns Hopkins with the M.D. degree in 1901 and, as Resident Pathologist (1901–03) and Director of the Ayer Clinical Laboratory (1904–1910) at the Pennsylvania Hospital, he pursued the studies in bacteriology and pathology which had caught his interest during his student days. There he began the investigation of antigen-antibody mechanisms which were of abiding interest to him throughout his career (16). In 1911, he left his position as Assistant Professor of Applied Medicine at the University of Pennsylvania for an associate professorship in Columbia University's department of medicine. In 1914, he succeeded his chief, Dr. Theodore C. Janeway, as Bard Professor of Medicine and Director of Medical Service at the Presbyterian Hospital. He held both posts until 1921. In mid-1922, he became Professor of Medicine and Physician-in-Chief at Johns Hopkins and there undertook a series of important investigations on hemorrhagic nephritis. During the Second World War, despite his 65 years of age and the additional teaching burdens which devolved upon him in the wartime absence of many of the younger staff members, he managed to supervise and participate in a series of studies on the effect of British Anti-Lewisite (16).

That Dr. Longcope was a life-long student of medicine is in part attested to by the fact that throughout his career he maintained up-to-date files on his "favorite" diseases—syphilitic aortitis, infectious mononucleosis, Hodgkin's disease, serum disease, hemorrhagic nephritis, virus pneumonia, and sarcoidosis—which contained reprints, case notes, miscellaneous clippings, photographs and personal observations. To these files he continued to add right up to the last weeks of his life (16).

Joseph Hersey Pratt (1872–1956), another Hopkins graduate in medicine (1898), also had opportunities to pursue his studies in the German universities, working under Krehl in 1902 at Tübingen and in 1908 at Heidelberg. Meanwhile, following his graduation from Hopkins, he began his professional career in Boston. His first positions were residencies in pathology at several of that city's hospitals; Instructor in Pathology at the Harvard Medical School; and, later, Assistant (1902–09) and Instructor (1909–17) in Medicine at Harvard and Visiting Physician to the Out-Patient Department at Massachusetts General Hospital (1903–13).

During this period, he became interested in tuberculosis. Finding it difficult to meet each of his clinic patients individually, he organized, in 1905, weekly classes for group instruction in the treatment of tuberculosis with such sidelights as prizes for those who had accumulated the most hours out-of-doors or gained the most weight (17). The innovation proved to be both popular and effective and 25 years later, as Physician-in-Chief at the Boston Dispensary (1927–31), Dr. Pratt extended the ideas inherent in these classes to the area of group therapy for emotionally disturbed patients. His "Thought Control Classes," designed to provide mutual encouragement and stimulation to such patients, were pioneer ventures in the field of group psychotherapy (17).

In 1929, Dr. Pratt became Professor of Clinical Medicine at Tufts Medical School and two years later, Physician-in-Chief of the New England

Medical Center which was created through the affiliation of the Boston Dispensary, Tufts Medical School, and the Floating Hospital for Children. Another of his outstanding accomplishments involved the dissemination of medical knowledge and the promotion of better medical care among New England practitioners (17). In 1930, he spent several weeks caring for a wealthy gentlemen named William Bingham, 2nd, who thereafter provided the financial backing for a program organized by Dr. Pratt and designed, in particular, to assist local physicians in the diagnosis of puzzling cases before referring them back for treatment. In 1937, on Dr. Pratt's sixty-fifth birthday, the cornerstone of the Diagnostic Hospital which bears his name was laid. Later, a surgical unit, a medical research laboratory and other buildings were added, all through the generosity of the "Bingham Associates." In the meantime, Dr. Pratt organized a program whereby family doctors were invited to spend a period of time in Boston observing and participating in the work of the Medical Center, and another in which able medical students were encouraged to spend a kind of "apprenticeship" as assistants to local physicians (17). These programs, as well as the "Thought Control Classes," have continued in effective use and are today a testimonial to Dr. Pratt's remarkable leadership and teaching abilities.

Wilder Tileston (1875–) did both his graduate and undergraduate work at Harvard, receiving the M.D. degree in 1899. He served his internship at the Massachusetts General Hospital and then held teaching positions at Harvard in Clinical Chemistry, Clinical Medicine, and Pathology until 1909. In that year, he went to New Haven to become Assistant Professor of Medicine at the Yale University Medical School, a position which he held until 1919 when he was advanced to the post of Professor of Clinical Medicine (1919–20). Subsequently he became Clinical Professor of Medicine. During these years he also held positions in several hospitals in New Haven and nearby towns.

These, then, are the nine men who directed the Society's debut. We have already gained some insight into the nature of the concerns which might have prompted them to take part in the new venture, but there is undoubtedly more to be discovered about their motivation. Why, we may legitimately wonder, did they agree that a new society was needed? What were the slightly "different aims" and "different membership" they envisioned? What was the nature of their apparent dissatisfaction with those societies "already in existence"?

It is evident from many of the recollections included in the preceding section that a major stimulus to the Society's birth was the fact that the well-established Association of American Physicians could not accommodate the rapidly increasing number of young men eagerly awaiting an opportunity to present their work before that much-respected body of elders. These young physicians no doubt attended the AAP meetings just as today much larger crowds flock to Atlantic City to listen to the scientific sessions of the Association, the Society, and the still younger American Federation for Clinical Research. Although the records of the Association do not attest to the fact, it is quite probable that the impetus for the formation of the Society came, at least informally, from within the AAP itself. Of the nine member committee which laid the plans for the Society's charter meeting, five—Drs. Capps, Christian, Edsall, Meltzer and Pratt—were at the time members or associate members of the AAP. While there was never any formal affiliation between the two societies, the founding of the Young Turks certainly had the dual effect of alleviating pressure for expansion on the Association and of providing a forum for its youthful disciples.

The Association's membership, in 1908, comprised largely the outstanding consulting internists of the day, most of them part-time professors as well as practitioners. A few men representing the basic sciences had been included to enable the physicians to keep abreast of developments in related fields. All were distinguished, top-ranking men.

Were they, perhaps, too distinguished? This is a view expressed by some Society members in accounting for the "revolt" of the Young Turks. The Association, they contend, had become a "very conservative and somewhat dull society, unaware of the tremendous advances being made in medicine." Its members were somewhat "set in their ways"—eminent men without a doubt, but "not very scientific." The new society was formed as a "protest" against these distinguished "stiff shirts." It consisted of "rebels and roughnecks"

who wanted a meeting wherein they could "get up and report real experimental results, not case histories."

These judgments represent a rather extreme point of view, and it should be noted that they constitute a hand-me-down version of the story.[5] Although it undoubtedly contains elements of truth, the degree of disesteem for their elders imputed to the first-generation Young Turks has probably been considerably exaggerated with the passage of time. Dr. J. Howard Means, the Association's official historian, does not believe that that organization's programs could legitimately be called "too stuffy and stale" during the years immediately preceding the Society's formation. Although the titles were generally broad enough so that any member was qualified to discuss any paper and the programs usually very clinical, Dr. Means points also to the fact that the older physicians were eager to keep in touch with the allied sciences and to maintain an up-to-date point of view.

The proper balance is probably struck by Dr. Cole (18) in the following account:

> I was much interested in the AAP to which I was elected when I was very young, and I had attended the meetings in Washington when I was still a student and intern in Baltimore. . . . I know that some of the members, especially Dr. Meltzer and some of the younger members, felt that the papers were not scientific enough, and some of the older members complained that they were too technical. . . . The limited membership of the Association made it impossible to elect all the younger men who manifestly deserved to belong. But I cannot recall that there were any acrimonious feelings or any spirit of rivalry exhibited.

The testimony of other charter members (2, 19) bears out the fact that the "insurrection" of the Young Turks was entirely good-natured. The new Society was formed not so much in protest against the old as in response to the needs of a younger generation which was beginning to use methods unfamiliar to its elders. Although the Society's organization, its structure, even the wording of its constitution reveal a hereditary relationship with the Association, the new organization at the outset exhibited distinct tendencies to depart from the ways of its parent. The following excerpt from a letter written by Dr. G. Canby Robinson (5), who was elected to the Society in 1911, suggests the difference in emphasis and outlook which was to mark the younger group:

> The idea of the Society was to bring together the men in the various clinics which were just being organized on a university basis and who were beginning to develop systematic research. It was a young crowd and was to be confined to the men who were active in clinical investigation rather than men who had become eminent in medical practice and teaching. The original idea was, I think, to have a one-day session of the acutal investigators the day before the AAP met, a plan that has been carried on to the present time.

Thus, in part, the difference was simply one of age—the Society was to provide a forum for young men whose time was still primarily devoted to research, whose energies had not yet been diverted into practice and teaching. In addition, however, Dr. Robinson notes that many of these young men were associated with the newly organized university clinics, As such, they were at the forefront of a far-reaching reform movement in medicine, a movement involving what Dr. Robinson (10) has characterized as "the intrusion of science into clinical medicine."

Significant components of this new outlook were the beliefs that clinical medicine was a science in its own right, that the investigation of disease was the legitimate concern of the clinician, and that the work of clinic and laboratory—of physician and basic scientist—ought to be more closely integrated and, in fact, combined in the person of an essentially new type of medical worker, the clinical investigator. Such beliefs had a number of important implications. The cultivation of a "research atmosphere" in medical schools and hospitals necessitated first, professors of medicine able to devote full time to research and teaching without the distraction of an extensive private practice; second, students and staff members trained in the methods and imbued with the spirit of science; and third, adequate laboratory facilities available to clinicians for the systematic study of clinical problems.

The realization of such conditions became the rallying cry of the new Society, but the reforms envisioned by its members were not to be instituted without a struggle. As partakers of the crusading spirit, the youthful Society members were very early designated "Young Turks" after

[5] The foregoing paragraph is a composite of statements made by several men who were elected to membership in the Society in the mid-1920's and whose information was based on what they had been told by older colleagues.

the "real" Young Turks who in 1908 startled the world with a revolt against Sultan Abdul Hamid II, deposed him the following year and, with nationalistic fervor, set about to establish a constitutional regime and institute sweeping reforms in the decrepit Ottoman Empire (20). Newspapers in 1908 headlined the visionary, albeit impracticable, tactics of these "young men in a hurry" as they were called, and someone drew the parallel between the Turkish revolutionaries and their counterparts in American medical circles.

The concepts for which the Young Turks stood were not widely accepted in 1908. In academic medical circles there existed a definite schism between the basic scientist and the clinician (21). The divergence between the two and the direction of change which was to heal the rift, is illuminated in the following passage by Dr. Means (13), in which he describes the situation at Harvard University where he began his own medical training in 1907:

> The transition from the preclinical to the clinical years (third and fourth) in medical school involved almost as great a change in direction as that from college to professional school. The teachers of the first- and second-year subjects and those of the third- and fourth-year subjects constituted two distinct groups with quite different viewpoints, and there was limited communication between them. The clinicians brushed off the preclinical scientists as mere laboratory men who knew but little of medicine, and the scientists looked upon the practice of medicine as largely unscientific guesswork. The whole manner of life of these two groups was different. The preclinical scientists were in the mold of college teachers on campus. They were full-time salaried people. There were no full-time clinical teachers in those days—or salaries either, for that matter. Small token honoraria were given by the university but nothing by the teaching hospitals. These men taught and attended hospital patients for love or kudos, or a mixture of the two, and made their livelihoods in private practice. Any degree of unanimity in a faculty so constituted I am sure was hard to come by. This gap was . . . later to be closed by the development of the "middle estate" in medicine—namely the full-time academic, salaried clinician.

The development of this "middle estate" in medicine, which was to take place largely during the first decades of the Society's existence and to be an important part of the lives of many of its members, called for a new type of clinical teacher —one who was research-oriented rather than practice-oriented (10). Essentially, the difference between the two was the difference between the German and British schools of medical teaching. At the time the Society was founded, the predominating medical influence in America was British as represented, for example, by Dr. William Osler, Professor of Medicine at Johns Hopkins University until 1905. It was to the British school, too, that the majority of the established "elders" of the American Association of Physicians, who combined teaching with practice, belonged. Many of the members of the younger generation, however, were strongly influenced by the medical traditions of Germany. Some of them had been fortunate enough to study at the university clinics of that country where medicine enjoyed a status equal to that of the other sciences, where its teachers devoted most of their time to instruction and investigation, and where research flourished. It was in this direction that the reformers of American medical education hoped to move and it was this tradition, with its emphasis on research in academic clinical medicine, which counted as a significant motivating factor in the founding of the Society (22). The German influence is particularly apparent in such men as Dr. Meltzer, Dr. Cole and Dr. Lewellys F. Barker who was elected to the Society in 1914 and who, in 1905, had succeeded Osler as Professor of Medicine at Hopkins. The difference between the older tradition in clinical teaching and the German-influenced ideas which were beginning to challenge it can perhaps be most clearly illustrated by a brief comparison of the Hopkins department of medicine under Osler and under Barker (10, 14, 22).

Osler was first and foremost an eminent physician and this fact was reflected in his conception of the aims of medical education and the role of the professor of medicine. Major emphasis was placed on imparting to students the techniques and skills necessary for observing and interpreting the manifestations of disease in patients; little on the investigation of underlying disease precesses. As Dr. Arthur Bloomfield (22) has put it: "Osler, I have been told, felt strongly that those things which are Caesar's should be rendered unto Caesar. In other words, laboratory work should be done in the pathological and physiological laboratories and not in the medical clinics by clinicians." Dr. Cole, who completed his medical training at Hopkins in 1899 and served under both Osler and Barker as Resident Physician, has described this attitude further (14):

> . . . (U)nder Dr. Osler the opportunities for careful observation were never better and the importance of

careful study of the more superficial aspects of disease never more insisted upon. But there existed in the clinic no great incentive to learn more about the fundamental nature of disease, and facilities for making the necessary investigation were lacking. The belief was general that medicine was basically different from biology or chemistry or anatomy and could only be studied by different methods. From time to time doubts about this point of view were expressed, but chiefly by workers in the underlying sciences and they usually held the opinion that the real investigations must be carried out by workers in their laboratories, since clinicians neither had the necessary time nor did they have the adequate training for these more complicated researches.

Dr. Barker, on the other hand, held that a primary function of the university department of medicine should be the encouragement of research and, accordingly, that the professor of medicine should be freed from the burdens of a private practice and allowed to devote his time to his own investigations and those of his staff (10). Although Barker's request that he be permitted to accept the professorship on a full-time basis was denied, he took important steps in the direction of organizing the department along research-centered lines. Shortly after coming to Hopkins, he established laboratories in his clinic for investigation in the fields of biology, chemistry and physiology and added to his staff clinicians with training in these areas. These clinical research laboratories differed radically, both in scope and in purpose, from the usual hospital laboratory which existed primarily for diagnostic and teaching purposes and for such routine work as the analysis of bloods and urines (10, 22). The new laboratories were designed to provide the clinician with the opportunity and the facilities for more fundamental study of the problems which confronted him on the wards. Dr. Cole was put in charge of developing the first of these research laboratories, that devoted to biological studies, and in it he began immediately to carry out investigations of typhoid fever which not only contributed significantly to an understanding of the nature of that disease (10), but which stood as indisputable evidence to the thesis that the scientific experimental method could successfully be applied by clinicians to the study of clinical problems. When, in 1908, Dr. Cole was asked to become the first Director of the Hospital of the Rockefeller Institute, he acquired a unique opportunity to give concrete expression to this point of view. In formulating plans for the new Hospital, he was insistent on the importance of building the wards in close proximity to the Institute laboratories and of including on his staff men who could bring their knowledge of the basic sciences to bear on clinical problems. His views on this subject, in striking contrast to those of Osler, are summarized by Dr. Robinson (10) as follows:

It was his (Cole's) opinion that the physical and intellectual barrier between the laboratory and wards then existing in many hospitals had seriously delayed the advancement of medicine in this country; he urged that the hospital be developed as a true research laboratory and expressed the fullest sympathy with the closest possible connection between the institute laboratories and the hospital. This relationship represented new and favorable conditions for the development of clinical medicine as a science.

Closely related to this new conception of clinical medicine was the question of the type of training appropriate to the aspiring clinical scientist, a point on which Dr. Meltzer had particularly strong convictions. It was his belief that training in one of the allied sciences of medicine, with extensive work in the laboratory, was as essential to the development of a top-notch clinician as was his training on the wards. That he differed from the majority of his contemporaries in this respect is brought out by Dr. Pratt (8):

Many of the older clinicians seemed to think it was a foolish thing for a young man who expected to become a clinician to engage in physiological or chemical studies in the laboratory, whether the observations were on patients or on animals. Dr. Meltzer thought it very important to encourage and assist young clinicians who were carrying on investigative work, especially in the laboratory, during their early years.

Dr. Meltzer was to stress this belief, and the broader attitude of which it was a part, when he addressed the Society as its first president. In his talk, entitled "The Science of Clinical Medicine: What it Ought To Be and the Men To Uphold It" (23), he directed attention to the shortcomings of investigative medicine in this country, outlined a vigorous campaign for its betterment, and exhorted his listeners to dedicate themselves to the struggle which lay ahead. The influence of the German tradition on the Society's ideals is clearly apparent in his statement. There could be no more appropriate summary of the considerations which gave rise to the Society's birth and the concerns that were to shape its future than the words of its founder and first president. Dr. Meltzer's address is herewith reprinted in full.

THE SCIENCE OF CLINICAL MEDICINE

WHAT IT OUGHT TO BE AND THE MEN TO UPHOLD IT

By SAMUEL J. MELTZER

The honor of the presidency of a society goes with the privilege of discussing some of the aims of that society. Such a privilege becomes a duty on the occasion of its first birthday. We have to show the specificity of our objects to justify the addition of a new medical society to the many already in existence. As your first president and as one of the instigators of the movement which led to the formation of this association permit me to discuss some of the problems to the solution of which this association wishes and hopes to contribute.

CLINICAL MEDICINE AS A SCIENCE

In the first place, I wish to discuss the problem of clinical medicine as a science. In years gone by, medicine was a unit and its leaders tried to master all its aspects. With the development of scientific methods and the growth of knowledge a process of differentiation took place. Heavy branches grew out of the stem of medicine, broke off and obtained an independent existence. Anatomy with all its dependencies broke away early, then followed physiology, pathologic anatomy, pharmacology and physiologic chemistry. Bacteriology tore off the branches of etiology and established itself as an independent growth. All these offsprings of medicine are now well established as pure sciences; they still closely affiliate with the mother-stem and are often designated as the sciences of medicine. That what is left of the old stem is clinical medicine. What is the character of this residuum? It is generally designated as the practice of medicine. I am not aware that any one has had the courage to call it a pure science. Those who do not like to call it an art say that it is an applied science. According to this view, the relation of clinical medicine to the sciences of medicine is that of technology to science in general. I am afraid that this conception of clinical medicine is widespread among those who are in the practice as well as among those who are in the sciences of medicine.

However this may be, I feel sure that such a view is logically erroneous and practically harmful. I wish to plead for an extension of the process of differentiation also to clinical medicine. I am of the opinion that clinical medicine as it exists now is made up of two constituents: one part has all the elements of a pure science and ought to be coordinate to the other pure sciences of medicine, and the other part is the real practice of medicine, an applied science which has many elements of an art. At present both parts are so closely interwoven that they present the appearance of a natural unit the splitting of which into two parts might appear to some as an impossible and undesirable process. I shall call to mind, however, that medicine made the same impression of an indivisible unit in the days when pathologic anatomy, physiology, etc., were inseparable constituents. To-day nobody doubts the necessity of an independent existence for the last-named constituents of former medicine. A considerable degree of separation of the science of clinical medicine from its practice is more desirable than even the separation of the coordinate sciences, because the requirements of sciences and practice are in a certain sense mutually antagonistic to one another and the simultaneous cultivation of both branches with equal attention is detrimental to the progress of either of them.

There is a fundamental difference between science and its correlated practice in

any sphere of human activity. Science wishes to attain truthful knowledge of the subject it investigates. It attains this end in the safest way when it is least sidetracked by any extraneous motives. The motive of utility obscures its vision, and haste retards its progress. Practice, on the other hand, avails itself of acquired knowledge for the purpose of attaining a certain useful end. The more reliable that knowledge is which it has to employ, the more safely will it attain that end. But the use of any part of that knowledge which is indifferent to the attainment of the useful end hampers the practical progress. In other words, practice gains its ends best the purer the quality and the smaller the quantity of the science which it has to use. Permit me to say that my advocacy of the separation of a clinical science from its practice is not actuated by any disregard for the practice of medicine. On the contrary, I entertain now and have always entertained a strong conviction that the efficiency of practice should be the supreme object in medicine. At the same time I feel sure that the efficiency of that practice will be best attained when the search for the knowledge which the practice has to use should be carried on in the same manner and by the same methods as are employed in the search for knowledge in other branches of intellectual activity. In other words, clinical research should be raised to a department of a clinical science and be theoretically and practically separated to a considerable degree from the mere practical interests, that is, separated so far as the various and variable conditions permit. It will be the practice not less than the science of medicine which will benefit by such a separation.

Now let us see what the domain of clinical medicine is and whether it contains problems which can be treated by the methods employed in pure science. The sciences of normal anatomy and physiology teach us the structures and functions of the normal body; pathologic anatomy tells us of the anatomic changes found in a body dead from one disease or another. To clinical medicine is left the study of the phenomena and their sequence as they occur in a living body during the entire course of a disease. It has to interpret these phenomena by known physiologic laws, to identify them with similar phenomena experimentally produced in animals and it has to correlate the phenomena observed in the course of a certain disease during life with the anatomic changes found at the postmortem examinations in case the disease has a fatal termination. It is also one of the tasks of clinical medicine to study the influence on the onset and course of phenomena of diseases which certain means and remedies may exert whose physiologic actions are well established. In other words, the domain of clinical research comprises the study of the natural history of diseases, their physiology and their pharmacology. Can there be any doubt that these studies constitute legitimate objects of a pure science? Objections may be raised, and will be raised in certain quarters, against the admission of the clinical science to the sacred inner circle of pure sciences on account of the impossibility in clinical medicine to verify the conclusions by the experimental methods. Assuming that this is an unassailable fact, what of it? Are the problems of biology, a science which enjoys an unassailable position in the councils of pure sciences, all or many of them provable by experiment? Or are the sciences of paleontology or geology amenable to an experimental proof? Yet nobody questions the scientific right of these branches of human knowledge. Furthermore, admitting that clinical research must necessarily remain less precise and its results less convincing than that, for instance, of physiology. But, then, physiology itself is again a great deal less precise than physics and chemistry and is certainly less irrefutable than anatomy. Does this throw a doubt on the claim of physiology to be a pure science?

As to the impossibility of verifying certain conclusions regarding human diseases by human experimentation, there is still this much to say. Each disease is an experiment which Nature makes on the organism. The very large number and the great variations of these experiments of Nature offer favorable opportunities for testing the theories made regarding the nature of these spontaneous experiments. Furthermore, clinical research should, indeed, be coupled with animal experimentation; any new point of view gained by observation in clinical medicine which cannot be verified on human beings should be tested by experiments on animals. By such a procedure not only clinical medicine, but also physiology may learn a good deal, for which the following facts from the recent history of medicine are classical illustrations. The recognition of myxedema as a special type of disease led to the understanding of the function of the thyroid glands, and the observation of the coincidence of pathologic processes of the pancreas with diabetes led to the discovery by clinical investigators of the glycolytic function of the pancreas. Finally, it should be borne in mind that there are lines of experimentation which may be carried out even on the sick human being without any detriment to him and which can be conducted with profit only by trained scientific observers, for instance some studies in pharmacology or in metabolism.

Among the tasks of clinical medicine there is one which is on the borderland between the science and practice of medicine, being perhaps of greater importance to the latter than to the former. It is the search for simple or complicated means by which established units of diseases may be recognized safely and perhaps also easily. In one word, it is diagnosis. Since this activity is not carried on with a purpose of adding knowledge, it would appear that it belongs rather to the department of the practice of medicine. Such facts can be established only by means of careful critical observations and it will be more to the purpose when at least the development of the principles of diagnosis should remain in the hands trained in careful methods of research.

After showing that there is a definite field for a science of clinical medicine the next question is who should be the men to carry on the research in this field, what should be their qualifications? In the first place, they must have a training fitting them to carry out investigations in conformity with the requirements existing in all pure sciences. They must not only be informed and trained in the other sciences of medicine, but must have carried on various investigations in one or more of these pure sciences, so as to become familiar with careful scientific methods and imbued with a scientific spirit. They will thus learn best how to shape a problem so as to make it amenable to a solution; how to marshall the consecutive steps of an investigation so as to bring out the reply to the question in hand. They will learn to avoid bias in the search, to apply criticism to the findings; they will learn not to trust only few facts, not to rejoice prematurely over findings and not to be disheartened by failures. They will thus acquire the habits and the tastes of the investigator, the scientist, which may then stick to them for life.

However, after all these preparations they must select clinical research as the main field of their scientific activity. Clinical science will not thrive through chance investigations by friendly neighbors from the adjoining practical and scientific domains. Such volunteer service which for the present is keeping up the cultivation of the unacclaimed region is most certainly very welcome. But the acclamation, cultivation and maintenance of a field of pure science of clinical medicine cannot be accomplished by chance services from volunteers; for such a purpose we need the

service of a standing army of regulars. The investigator in clinical science must devote the best part of his time and intellectual energies to the cultivation and elevation of this field just as the physiologist does in his domain—or at least as he ought to do. For the sake of elucidation let us compare the relations of a science of clinical medicine to its practice with the relations of science to technology in any part of physics or chemistry. Let us take electricity as an instance. In the electrical industry there is a large corps of electrical engineers who are bright, inventive and on the alert to make practical use of any newly discovered principle in general. Some practical engineers manage to make valuable contributions to the theoretical knowledge of their branch. Nevertheless the science of electricity is in the hands of pure scientists who are setting the pace of progress in the correlated practical branch. It is the discovery in pure science which makes great inventions possible. It was, for instance, the purely scientific demonstration by Hertz of the existence of electric waves that gave the impetus to and made the invention of wireless telegraphy possible. The following is a quotation from the presidential address of Professor Nichols before the Association for Advancement of Science:[1] "Communities having the most thorough fundamental knowledge of pure science will show the greatest output of really practical inventions." This is surely true also for medicine. And let me say to you that the Hertz's, the Roentgens, the Bunsens, the Emil Fischers, and a host of others, who are the real creators of knowledge and invention, are satisfied to live on comparatively small salaries, while it is within their easy reach to make twenty times the amount of their salaries as consultants, if they would be willing to give up a part of their time which they devote to investigations in pure science. Now, the leaders in practical engineering in electricity surely compare favorably with the leaders in the practice of medicine with regard to the completeness of their knowledge in the respective spheres. While thus the search for knowledge in electricity is carried on by men devoting themselves to pure science, the search for knowledge in clinical medicine is left in the hands of men who devote most of their time and energies to their practice and to the golden fruit it bears. Under such circumstances, how shall we expect to find the progress in science and practice of medicine keeping abreast with the striking progress to be met within other branches of science and practice?

A few years ago I tried to fix the responsibility for the shortcoming of internal medicine in physiology, because this science is keeping aloof from medicine and its problems.[2] Anatomy, which gave its undivided attention to the human structures, prepared an excellent basis for a successful surgery, the problems of which are essentially mechanical in character. The problems of clinical medicine are essentially of a functional character, and the enlightenment which it requires must come from physiology. This science, however, developed a tendency to keep at a distance from medicine on account of the inexactness of the latter. There is an improvement to be noted in this regard; there is a growing tendency in physiology nowadays to pay greater attention to medical problems. However, even if physiology and other related medical sciences would devote all their time and energy to the study of purely medical problems, it would not bring about a radical solution of our perplexing problem. Neither will this be accomplished by the creation of chairs for experimental medicine. The regeneration or rather creation of a science of clinical medicine must come from the innermost of medicine itself. It is true, as stated

[1] Science, Jan. 1, 1909, p. 5.

[2] Congress of Science and Art, 1904, v. 395; chairman's address.

before, that the men who are to tackle these problems must have a thorough training in the sciences allied to medicine, but the center of their activities must be within clinical medicine itself. They must have a bringing up within medicine, their minds must have been filled up with thinking, worrying, brooding over practical and theoretical problems of clinical medicine.

Let me recapitulate. There is a necessity for a differentiation of clinical medicine into a science and a practice. The clinical science has well-defined objects and is a most important department. It must be managed by a set of men specially trained for and almost exclusively devoted to that task. Without the development of such a department of clinical science the efficiency of the practice of internal medicine will lag behind, no matter how progressive the allied sciences of medicine are and how great their efforts to be useful to medicine may be.

THE CONDITIONS AS THEY ACTUALLY EXIST

In conjunction with the foregoing considerations let me glance at the conditions of clinical medicine as they actually exist here and abroad. I may state that neither abroad nor here do we find a separate class of investigators confining their activity solely to the domain of the science of clinical medicine. Everywhere science and practice of clinical medicine go hand in hand. We may, however, admit at the outset that there is an enormous difference in the status of medical research between abroad and here. Let us take the development and the present status of clinical medicine of the German-speaking countries as an illustration of the conditions abroad. We shall notice that there the mother stem of medicine managed to retain a scientific aspect despite all processes of differentiation.

When for the first time pathologic anatomy broke away completely from medicine and was taught by Rokitansky at the University of Vienna as an independent branch of medical science, we find that at the same time and at the same place the celebrated Skoda was teaching the remaining trunk of clinical medicine as a natural science, constructing the methods of auscultation and percussion on a sound basis and establishing a firm connection between the *intravitam* diagnosis and the post-mortem findings. The purely anatomic tendencies of that celebrated school of medicine which unfortunately gave birth to nihilism in medicine, was soon curbed by the appearance of Virchow on the medical scene in Berlin. While also working for the establishment of pathologic anatomy, gross and histologic, as an independent natural science, he manifested throughout his long life a deep interest in the development of clinical medicine on sound lines. At the very beginning of his brilliant career we find him stating (in the prospectus to the first volume of his *Archiv*) that practice of medicine should be an applied science and the ideal of the science should be a complete pathologic physiology. The great clinical contemporaries of Virchow strove for a similar ideal. Frerichs introduced chemistry and Ludwig Traube the methods of experimental physiology into the domain of clinical medicine. I shall not dwell here further on historical details. I shall only say that clinical medicine in Germany remained true to this ideal throughout all these years of marvelous growth of medicine. The exponents of clinical medicine in Germany are the flag bearers of pathologic physiology. The contemporary clinicians master the immense knowledge and manual skill which contemporary clinical medicine demands of its disciples, and nearly all of them are themselves diligently adding new facts to the store of medical science. I do not need to tell you the particulars. Some of you know from personal experience such master clinicians like Müller, Krehl, Kraus,

Minkowsky, and a good many others like them. However, the standard of medicine is kept high not only by the heads of university clinics; the various members of their staffs, heads of hospitals and their staffs and a host of volunteer workers are continually digging for new fruit in the soil of clinical medicine. The medical literature is full of original, first-rate work in the science of clinical as well as of experimental medicine contributed by men whose main abode is clinical medicine.

Now there is in Germany no special chair for the science of clinical or even experimental medicine. All these contributors to medical science are in a sense simply medical practitioners. What is the secret of their scientific success? It is simply this: to these men science is the first and practice the second object of their life. To most of the leaders of medicine in Germany the tasks of gaining and diffusing knowledge receives their first attention and the consultation practice comes in last. Again, these very leaders before they have acquired their commanding positions spent many years of their life in preparing themselves for it; in training in scientific methods, in acquiring knowledge in the allied sciences and in the science and practice of clinical medicine, living meanwhile on small incomes, having no private practice at all or having a practice too small to interfere with their life ambition. You cannot lead such a life unless you are possessed by idealism and a scientific spirit. That is the key to their success. And when these men become teachers, they cannot fail to impart this ideal spirit to their students. That is the way they themselves received it. But they received it also in the institutions of their early education, where sport and habits of millionaires' sons are not the factors which form the character of the youth. They received it from the people among whom they grew up, among whom the worth of the individual is not measured exclusively by a gold standard. They received it from the care and encouragement of the government which, no matter what its political ideals may be, never loses sight of the fact that the cultivation of science is one of the greatest assets of a people. They received it in the traditions of medicine of their country; even before the new era when medicine was all in a cloud of so-called natural philosophy, only the substance of medicine was all wrong, its spirit was all right.

That is the situation abroad. Now let us look at the development and the present status of clinical medicine in this country. At a glance one observes with amazement that nearly all the factors which favored the development of a scientific spirit in Germany are absent. However, I shall not try to enumerate all the shortcomings with which the development of medicine in this country had and still has to contend. There is no educational advantage in doing it and you are all familiar with the situation. On the contrary, I take pleasure in pointing out the encouraging fact that, despite all the difficulties, medicine in this country gradually rose to a commanding position in many respects. It is an instructive and stimulating fact that the progress of medicine in this country was carried on through the efforts of a few high-minded men or groups of men who at various times transplanted the spirit prevailing abroad into this country. I shall, of course, not attempt to enter into the particulars of the history of this progress. I wish to refer briefly, however, to a few phases in this history. In the first important phase, clinical medicine in America received an elevating impulse through the activities of a group of medical men who continued their studies in France, where they came especially under the influence of the famous clinician Louis. Statistical methods of observation of diseases and diagnosis by auscultation and percussion became, then, the chief characteristics of internal medicine in this country. During the second period, young

American physicians pursued their postgraduate studies in Austria and Germany. They brought home a knowledge of gross and minute pathologic anatomy and an aptitude for the use of the microscope. Pathologic societies were started and the teachers of medicine began to give separate lectures on pathologic anatomy and to instruct in the use of the microscope as a clinical aid. The studies and teaching of these new subjects gave at that time a scientific flavor to clinical medicine. The last period covers about the three last decades with the contradictory results of which we are confronted to-day. Again, medical men went abroad for postgraduate studies and perhaps in larger numbers than ever. But what this high class of young medical men studied there now was no longer in the direct interest of clinical medicine; they trained themselves in the intricacies of the sciences of medicine. The results of the studies of this period were again of great benefit to medicine in this country; in fact, they were of greater benefit than ever. In a comparatively short period the special sciences of medicine, thanks to the activity of a brilliant group of men, have gained a strong foothold in this country; special chairs and laboratories were created in many schools of medicine for pathology, physiology, bacteriology, pharmacology and physiologic chemistry. The work which is being accomplished now in these laboratories compares favorably with the work done in the countries with older scientific traditions and much more favorable surroundings. However, by this very departure clinical medicine itself suffered a visible retardation in the development of its own department. I discuss this phase not with pleasure, but the truth must be said. To my mind it is a fact that the leading clinicians of this generation do not compare favorably with their predecessors in this country, not to speak of a comparison with the leaders of clinical medicine in other countries. One of the reasons for the retardation in the progress is the loss to clinical medicine of the brainy men who now devote their energies to the pure sciences of medicine. Another reason may be found in the fact that by giving up pathologic anatomy and microscopy to special departments clinical medicine lost that part which has given it formerly its scientific character. An evident drawback to the progress of internal medicine in this country is the fact that its teaching at the present time is mostly still in the hands of men who received their medical training nearly exclusively in this country and at a time when pathologic anatomy was the exclusive basis and auscultation and percussion practically the exclusive methods of diagnosis. But in the last twenty-five years internal medicine changed its aspect greatly. The men who want to teach the medicine of to-day must have an education and training radically different from those which were customary and sufficient in former years.

However, the greatest evil of them all is the deplorable fact that in most instances internal medicine is taught in this country by very busy consultants who can give only a small fraction of their time and mental energy to this one of the side issues of their busy lives. Teaching medicine and furthering its science is a very serious business which ought to be carried on by men who are ready to devote all or most of their time to it.

Here are problems for you to solve. The progress of clinical medicine in this country has to be accelerated again. We might reach the level which we have seen to exist abroad. But we ought to strive to get still higher; in this field lack of modesty is a stimulating virtue. Look at the allied sciences of medicine, the level of which they have reached in a comparatively short time without any tradition and only through the activity of a small group of high-minded men. The progress of medicine in this country was always carried on by the few and not by the masses.

Clinical medicine must reclaim some of the brainy young men who were enticed by the sciences of medicine. You represent a new generation. You have had an education and a training in modern medicine and the allied sciences. You have a training in investigation, the constitution requires it of you. The constitution which you have adopted shows your spirit. Among its objects is "the cultivation of clinical research by the methods of the natural sciences and the diffusion of a scientific spirit among its members." But you will accomplish more; the constitution imposes on you the obligation to be active in the diffusion of the principles of the society particularly among the students who come under your charge. I would recall this obligation to the minds of the members who were called within this year to chairs of medicine at influential places. The constitution does not keep you down exclusively to science, but let me tell you: beware of practice. It is a bewitching graveyard in which many a brain has been buried alive with no other compensation than a gilded tombstone.

One last word. The men whom you now see sitting on the bank left behind while the boat of progress swiftly glides away with fresh winds and under fresh sails were themselves in their youth passengers of similar boats and cut faces at others who were left behind. Be generous to them, but do not repeat their mistakes. The secret is: never leave the boat.

II. FORMATIVE YEARS, 1909–1929

Dr. Meltzer's "New Science". . . .

The early years of the Society were characterized primarily by the serious work of attacking the problems outlined by Dr. Meltzer and striving to realize the purposes set forth by the constitution. Society members went about these tasks diligently and, for the first few years, with little to distract them.

The first annual meeting was held on May 10, 1909. Like the charter meeting, it took place at the New Willard Hotel in Washington. Subsequent meetings, prior to 1917, were usually held at this hotel when the Society met in Washington and at the Marlborough–Blenheim when in Atlantic City.

Fifteen of the Society's 31 members were present for this first meeting. With Dr. Meltzer in the chair, a number of routine business matters were dispensed with—Treasurer Howland reported a balance of $109.70 on the Society's books; officers for the forthcoming year were elected; a resolution on the death of one of the charter members, Dr. J. Dutton Steele, was passed; and new members were elected. It is interesting to note that this first meeting was the only one in the Society's history at which all nominees recommended by Council were not rubber-stamped into membership. Of a total of 20 nominees, eight were endorsed by Council, but of these, only seven received the requisite number of votes from the Society as a whole.

Following Dr. Meltzer's stirring address, the Society turned to the serious business of reporting the type of results which would further the causes he had outlined. The 12 titles on this program were as follows:

1. The blood pressure in tuberculosis—Haven Emerson
2. A modification of the Riva-Rocci method of determining blood pressure for use in the dog—Janeway
3. The effect of compression of the splanchnic arteries upon the systemic blood pressure—Longcope
4. Blood pressure changes following reduction of the renal artery circulation—Janeway
5. Miliary tuberculosis of the skin—Tileston
6. The sensory symptomatology of the facial nerve—Hunt
7. A study of experimental anemia—Pratt
8. Observations on metabolism in cases of muscle spasms due to heat—Edsall
9. The relation of atrophy of the pancreas to glycosuria—Pratt
10. Some further observations on experimental nephritis—Christian
11. Some experiments upon the possibility of catheterizing the pylorus in dogs—Sailer and Torrey
12. Respiration by continuous intrapulmonary pressure without the aid of muscular action—Meltzer and Auer

Each of these presentations was discussed, no doubt in considerable detail and with frankness, by the members. Although the minutes do not record the particulars of what was said, the participants in the debate over each paper are noted and, after nearly all presentations, several men were ready with comments. In this respect, the first five meetings of the Society must have been particularly uninhibited, for it was not until 1914 that the first inevitable limitations on the scientific program became necessary.

At that meeting—the sixth—the chairman announced that due to the length of the program (29 papers were scheduled), presentations were to be held to 10 minutes, discussions to five. In addition, the Society approved a Council recommendation restricting the number of papers to be presented on succeeding programs to the first 30 received by the secretary, the remainder to be read by title only. This curtailment was not, however, imposed without comment. After hearing the Council suggestion, Dr. Meltzer rose to speak. He moved adoption of the proposed limitation, conceding that for the present it was necessary and, as a temporary measure, acceptable. But, he continued—and we can surmise from the secretary's notes the fervor with which he spoke—the Society should consider changes of a much more radical nature for the future. There was no reason, he argued, why its meetings should be limited to a single day. He noted that membership had increased to more than 70 and that it was bound to increase still more. "Why," he implored, "should the Society continue to act like

an appendix to the AAP? It can meet at any other time; it can meet for two days; and it can have more papers!"

Despite his urgings, the Society apparently did not even consider such possiblities until 1921, and then it was only to reject a suggestion for a two-day session. Nevertheless, the 1914 discussion underlined what was to be a continuously troublesome issue: the problem of accommodating more men and more papers while at the same time guarding high standards through the exercise of selectivity in membership and programs. Such problems did not, however, become really pressing until a somewhat later period.

Growth during these early years was fairly slow, but steady. Seven new members were elected in both 1909 and 1910, and five in 1911, bringing the total number to approximately 50 by the time of the fourth annual meeting. In the spring of 1911, a letter was sent to all Society members urging them to exercise more diligently their right to nominate worthy men for membership. As a result, nominations jumped considerably and 14 of 26 candidates were elected in 1912. By 1916, membership had increased to just over 100 and, again, limitations on the program became necessary. This time, the number of papers to be read was reduced from 30 to 25, still to be presented in the order in which titles were submitted to the secretary. Neither this ruling, nor the earlier one, worked a great hardship in actual practice. Through 1920 the programs averaged about 27 papers and frequently, due to absences, fewer were actually presented at the meetings.

Perhaps indicative of the Society's complete preoccupation with the pursuit of knowledge during these years was the fact that all of its early presidents chose to bypass the "speech-making" prerogative of the office and, instead, devoted their opening remarks to a discussion of their own experimental work. Until 1917, with the exception of Dr. Meltzer's talk, the "presidential address" was simply the first paper on the scientific program.

Abstracts of papers read before the Society were published, for the first years, in the old Boston Medical and Surgical Journal (now New England Journal of Medicine) and in the Journal of the American Medical Association, and reprints were usually distributed to members. In 1914, the question arose as to whether the Society should arrange to have its proceedings published privately at its own expense. Dr. Meltzer moved the adoption of this proposal, urging the chair to appoint a committee to investigate means of implementing it. He urged the publication not only of abstracts, but of discussions as well, arguing that higher dues to meet the added, expense would be well spent for really good transactions.

Dr. Christian countered, expressing opposition to "expensive, extensive transactions," which, in his opinion, were "a sort of burial ground of papers." The private printing of brief abstracts and discussion summaries was acceptable, but if the proposal meant the regular publication of elaborate transactions, he viewed it with alarm. He was particularly concerned with keeping the dues low (they were $3.00 a year at that time), contending that a higher assessment would be hard on men just beginning their careers. He concluded by remarking on the semantics of the issue: "If the transactions are to be put out in abstracted form," he said, "it might be well to call them something else, as they would scarcely be worthy of being dignified by the title of 'transactions.'"

To this outburst, Dr. Meltzer replied by saying that Dr. Christian's points were the very reason a committee was needed. Forthwith, his original motion carried and a committee consisting of the two protagonists and Dr. Roger S. Morris was appointed. The following year, the committee reported, in somewhat inverse form, as follows:

> The Committee appointed by the President at the last annual meeting (1914) reports that it considers it as desirable that the American Society for the Advancement of Clinical Research shall henceforth publish a volume containing the transactions of each annual meeting, which would embody fuller information than hitherto, of the papers presented and the discussions which followed, a volume which should be accessible through libraries, etc., and which would lend significance and standing to the Association.
>
> The Committee recommends further the appointment by the present president of a new Committee which, after listening to a discussion by the Society, should "consider the proper means for accomplishing this object."
>
> (Signed) In favor of the above: S. J. Meltzer
>
> Opposed: H. A. Christian
> R. S. Morris

There was no discussion on the matter from the floor and, with two members of the committee in opposition, the Chair ruled that the Society's proceedings would continue to be published as before. A complete list of sources of Society proceedings is included in Dr. J. Harold Austin's history of the Society (24).

In 1916, the name of the Society was shortened from "American Society for the Advancement of Clinical Investigation" to its present form. The minutes state simply that in 1915 President Cole asked for suggestions for a new name, the old being considered somewhat unwieldy. Two possibilities, each in the form of a constitutional amendment, were offered: "American Society of Internal Medicine" and "American Society for Clinical Investigation." The latter alternative was adopted, apparently with neither discussion nor dissent, the following year.

Perhaps the change was indicative of the fact that clinical investigation no longer required quite such active "advancement." In any case, the year 1916 marked the end of the Society's initial phase. Its life during the succeeding period did not differ radically, but it had become a going concern and changes were inevitable. As far as meeting places were concerned, 1916 marked the end of the New Willard-Marlborough Blenheim days, the advent of the "Traymore era." Although the Society remained a close-knit organization, 1916 marked the end, too, of a kind of intimacy manifested in part by the very personal nature of the records kept by its early secretaries. And finally, with America's impending involvement in the First World War, 1916 was, perhaps, the last year in which physicians were permitted the indulgence of single-minded devotion to the "advancement of clinical investigation."

. . . . *And How It Grew*

In 1917, the Society turned its collective attention for the first time to problems of a medico-political nature by adopting two resolutions pertaining to the war. The first, introduced by Dr. Leonard G. Rowntree, was in response to the National Research Council's call to arms. It read as follows:

> Whereas, the National Research Council invites the cooperation of educational institutions in the promotion of research at this critical period in our national progress—
>
> Be it Resolved that we, the American Society for Clinical Investigation, hereby offer, through the President of the Society, our services and heartiest cooperation to the National Research Council on the lines of investigation which it considers most needed at the present time.

This was followed up two years later when Dr. Christian, then president of the Society, reported on a meeting of the National Research Council's Division of Medical Sciences of which he was Resident Chairman. In 1922, Dr. Means was appointed as the Society's representative to the National Research Council and thenceforth unto the present day the Society has maintained a somewhat "off-again, on-again" relationship with the Council.

The second resolution, probably the Society's one and only contribution to the cause of prohibition, is rather amusing in the light of a later day. At the time, it was in the direct line of patriotic duty. Introduced by Dr. Edsall, it read as follows:

> Resolved, that in the critical condition of the world's food supply, we consider it desirable that the manufacture of alcoholic beverages or their importation into this country be prohibited for the duration of the war and for at least one year thereafter.

It was passed, though apparently soon forgotten. As one member put it, "No one talked about prohibition when I got in in '22!" Be that as it may, everyone talked about prohibition in 1917. On the national scene, the anti-alcohol crusade had begun back before the turn of the century and by the time war was declared, two-thirds of the states had succumbed to its advance (25). The ease with which liquor could pass into these dry states from the wet areas led to a rising demand for national prohibition and in 1913 Congress passed the Webb-Kenyon act, penalizing shipment of alcoholic beverages into states where their sale was prohibited. When the United States entered the war, a nationwide ban on the sale and manufacture of all intoxicants was enacted ostensibly, at least, on grounds of national economy and efficiency. Whether "the critical condition of the world's food supply" actually had anything to do with the Society's action is open to speculation. But it was as likely a reason as any.

In addition to these two instances of response to national duty, another outward-looking feature

appeared on Society programs. This was the new style presidential address. In 1917, Dr. Haven Emerson departed from the pattern set by his predecessors and delivered a talk entitled "The Community as a Patient Needing Clinical Investigation." The following year, Dr. George Blumer addressed the meeting on "Medical Education in Relation to the War." From that time forward, with occasional exceptions, Society presidents have put themselves in a "watchdog" role, commenting on current conditions in clinical medicine in general and within the Society in particular, warning of potential pitfalls, and pointing to areas wherein changes are needed. The end of the First World War and the Society's tenth anniversary provided the point of departure for Dr. Christian's address entitled "The Work of the Medical Clinics" given at the 1919 meeting.[6]

The Society's first decade, Dr. Christian felt, could be regarded with satisfaction. "Gentlemen," he said, "much of what Dr. Meltzer outlined has been accomplished and this Society and its members have had a large share in bringing about the change." He continued, however, with a warning against complacency:

> To readjust ourselves to pre-war conditions is not easy; even to do this should not satisfy us; improvement in medical conditions alone should satisfy us. . . . This Society exists primarily for the encouragement of investigation. We must take counsel as to how this can be accomplished in the best possible way. . . . As chiefs of clinics what should be our attitude toward our junior men? Why have we not in the past developed better men in our clinics?

In presenting his own answers to these questions, Dr. Christian cited selfishness on the part of the department head as the primary cause of failure. Although it was important that the chief be responsible for work issuing from his service or laboratory, Dr. Christian looked with extreme disfavor on the department head who consistently took more than his share of the credit for such work. He stressed the importance of giving assistants freedom in their investigations, of encouraging the development of independent thought. On this score, he said:

> Personally, I have never cared for the method of outlining an investigation for an assistant, and when that is finished, outlining another. In the long run I think it is better to let the man drift in his work for awhile until he can see a problem for himself. . . . This is rather a kill or cure method, but after all is it worthwhile for the man without imagination and curiosity to investigate? I believe not; he has a place and a work to do but it is not investigation. To lead him to think he will succeed in investigation is doing him a wrong.

Ten years earlier, Dr. Meltzer had spoken of the need for clinical investigation to reclaim some of the "brainy young men" who had been lured away from medicine into the laboratories of the allied sciences. In this light, the following passage from Dr. Christian's address is rather interesting:

> Our recent development in the clinics (has) worked to weaken the fundamental sciences by taking from them a valuable group of workers, namely, those planning a career in clinical medicine. I believe it would be wise to return some of these for a year or so of work in these laboratories during the course of their training for internal medicine. To do so would be helpful both to the fundamental sciences and to clinical medicine.

The growth in the number of workers engaged in clinical research had, indeed, been rapid during the Society's first decade of existence and it was to become even more rapid during its second.

The years between 1910 and about 1935 witnessed striking advances in the status of investigative medicine and a veritable revolution in American medical education. The birth of the Society was nearly simultaneous with that of another institution destined to be of far-reaching significance for the future of American medical research—the Hospital of the Rockefeller Institute. Opened in October, 1910, the Rockefeller Hospital was the first in the country to be built especially for, and devoted entirely to, clinical investigation. In the years following its establishment, it exerted a profound influence on the modernization of academic medicine, serving as a model for the clinics then being established or reorganized on a full-time basis in universities both here and abroad and as a training ground for the men who were to staff them. Indeed, this was one of the avowed aims of its first director, Dr. Cole (14):

> As soon as the work was under way I realized that owing to conditions then existing in medical teaching the hospital should have at least one other function beside the investigation of disease. The idea of so-called university departments of medicine was in the air and it

[6] A copy of this address, which was never published, was obtained through the courtesy of Dr. C. Sidney Burwell.

was evident that this idea would soon reach concrete expression in a number of places. The new hospital appeared to be the logical place in which leaders of this new movement could be trained and be given opportunities to work and be fired with the spirit of investigation which could thus be disseminated throughout the projected clinics. It seemed that the hospital should not adopt a policy of "splendid isolation" but should play its part in the reorganization of medical teaching in this country.

That it succeeded admirably in this role is evidenced in part by the fact that in 1938, of the 179 persons who had been on the Hospital staff during its 28 year existence, 112—62.5 per cent—occupied full-time academic positions and many more had university affiliations of one kind or another (14).

The full-time system in clinical teaching was initiated in the United States with its establishment at Johns Hopkins in 1914 (9, 10) after Acting President William Henry Welch had obtained from the Rockefeller Foundation's General Education Board a grant sufficient to endow full-time chairs in the departments of medicine, surgery and pediatrics. It was instituted, however, only after a considerable battle between supporting and opposing factions of the medical faculty to win over the Hopkins Board of Trustees. Dr. Barker was among the foremost advocates of the full-time plan. When he had been called to succeed Osler as professor of medicine in 1905, he had petitioned the Board of Trustees to allow him to accept the position on a full-time basis but his request had been denied and, like his counterparts of that day, he had been forced to divide his time between university responsibilities and a private practice. In 1914, after the climate had changed, he was offered Hopkin's first full-time clinical position but by that time his practice had become so extensive and so closely integrated with those of other Baltimore physicians that it would have been difficult to abandon (10). It was left to his successor, Dr. Theodore C. Janeway, to become the nation's first full-time professor of medicine. To do so, he gave up a lucrative practice in New York. Dr. John Howland, like Dr. Janeway a charter member of the Society, was Hopkins' first full-time professor of pediatrics.

During the ensuing years, although the full-time system caught on and began to spread to other institutions, it encountered rough going. Perhaps the major point of contention was whether or not full-time teachers should continue to see private patients. The argument in favor of their doing so was based partly on the desire to supplement academic salaries, partly on the view that the teacher of medicine should retain contact with the care and treatment of patients beyond that connected with his duties in the university clinic. The full-time system as established at Hopkins (and at such other institutions as Washington, Rochester and Yale Universities where Rockefeller Foundation grants supported clinical chairs) carried with it the understanding that full-time staff members would not accept fees from the treatment of patients which they might undertake on their own (10, 21). Such fees, rather, were to be used by the departments, a stipulation designed to assure that the attention of the now-salaried clinicians would be focused exclusively on their university responsibilities—teaching, care of patients in the clinics, and research.

When Drs. Edsall and Christian established full-time clinical teaching on a firm basis at Harvard University, they developed certain variations on the Hopkins plan. Of the issues involved and of the "Harvard full-time system," Dr. Means (13) has written the following account:

. . . A policy question arose about just how much private practice the so-called full-timers should be allowed to do. Edsall consulted the trustees of the hospital about his own case, and all he could ever get out of them was that they considered him a gentleman and they hoped that he thought they were! The policy finally arrived at was that the amount of private practice should not be more than was consistent with doing a fair measure of full-time work. It was more a moral than a legal issue. Through the years it has undergone some modifications. How the private practice of the full-timers was to be paid for was also a problem. At Johns Hopkins Hospital—where, with abundant aid from the Rockefeller Foundation, the major clinical departments had been put on a strictly full-time salaried basis (staff members) were expected to live on their salaries. They could take care of patients privately, but the fees collected for such service went to the hospital and were applied to departmental budgets.

Edsall considered such a policy unsound. Inherent in it was the danger that the full-time staff could be exploited by the institution and forced to make a substantial contribution to the departmental budget by caring for private patients. It was possible, although not inevitable, that such a system could lead to serious encroachment on the academic services of the full-time people. He ar-

ranged, therefore, that the full-timers collect their own fees for the small amount of private practice (they) performed. This policy became known as the "Harvard full-time system."

During the 1920's, the adoption of the full-time system was greatly accelerated, and of the situation as of about 1932, Dr. Robinson (10) has written:

> Medical education in this country seemed to have become mature. The great medical development of Columbia University and the Presbyterian Hospital in New York, opened in 1928, was the outstanding medical center of the new type; the New York Hospital-Cornell Medical Center began its operations in 1932. In the meantime, new schools had been created at the University of Rochester and at Duke University. The medical schools of Vanderbilt and of the University of Chicago had been reorganized along the new university lines, and the development of clinical departments with full-time teachers was well under way.

Such developments did not come, however, without creating new problems in the realm of academic medicine. One of these was the subject of an address entitled "What Should We Do With a Harvey or a Laennec?" given in 1927 by Society President Eugene F. DuBois (*26*).

"With the tremendous growth of our medical clinics and medical schools," Dr. DuBois said, "we are in danger of being swamped in this process of expansion." He traced the hypothetical course of the clinical careers that a young William Harvey or a young René Theophile Laennec might follow in current America, lamenting the probability that, after an initial period in which they would be allowed to devote full time to research, their remarkable talents would be dissipated by increasing administrative, teaching, and consulting responsibilities. "What would become of the discoveries that made Harvey the founder of modern physiology or the pathological studies that made Laennec the founder of modern clinical medicine? How much time would be left for quiet work and contemplation?" Dr. DuBois asked. He continued:

> The situation would not be serious if it only meant the loss of the original investigations of a few such men. There is a more serious loss; that is the gradual atrophy of the research spirit through disuse. The true research spirit can be maintained only by means of personal contact and participation in the experiments. The head of the clinic is seldom allowed enough time for that study and work which alone will keep him the best informed man in his own particular field. The university scatters his energies over a broad expanse, part of which is as sterile as the Desert of Sahara.

Dr. DuBois attributed these failures to "the desire for bigness on the part of our universitites," and concluded, with respect to Harvey and Laennec, that "after a good look at the present conditions they would realize that they would be happier in their own small, inefficient, but comfortable, centuries."

The infiltration of a "mass-production mentality" into the realm of science was enlarged upon by Dr. Robert F. Loeb (*27*) in 1936. In considering the merits of the two general approaches to research—individual effort vs. the more recent "research project"—Dr. Loeb cited the experience of the past in upholding the view that it was the work of the creative individual, motivated by intellectual curiosity and capable of critical thought and imaginative experimentation, which offered the greatest hope for progress. Of the research project, he said, on the other hand:

> (It) demonstrates in its point of view total ignorance of the means by which science has advanced. It attempts to force progress through regimentation. . . . It stifles the untrammeled play of imagination. . . . It is not the product of creative thought, but is usually initiated by energetic and misguided promoters inspired by the effectiveness of mass production in big business. . . . In other words, it assumes that scientific knowledge can be bought, and that dollars give birth to ideas. The research project in clinical medicine has proven consistently barren and has resulted in the disbursement of funds which, if applied to the endowment of individual investigators or university departments where creative thought is fostered, may reasonably be expected to further the progress of science.

The expansion of clinical teaching and research which gave rise to such outcries made its presence felt in the Society's swelling membership ranks and plethora of papers as well. During the years following World War I, the problem of increasing numbers demanded almost annual attention. For the first time, the principle of selectivity was introduced in making up the program. Previous limitations, it will be remembered, involved setting an arbitrary quota on the number of papers to be read and filling it on a first-come, first-served basis. In 1920, Council recommended that preference on future programs be given to members who had not presented a paper the previous year.

This, however, did not make any assumptions about the comparative merits of the papers thus presented. In 1922, under increasing pressure to accommodate more titles, the Council took cognizance of the fact that the earlier method of making up the program did not necessarily result in the best selection. The Society voted that in the future selection would be left to the discretion of the president and, to assist him in his choice, abstracts were to be required. In 1924, restrictions having to do with qualifications to be met before a paper would be considered were introduced. The rulings passed that year provided that 1) no paper was to appear on the program unless one of the authors was a Society member; 2) except in the case of joint authorship, each member was limited to one paper; and 3) prior publication barred a paper from the program. These rulings were amended in 1931 to allow nonmembers to read papers if they were introduced by a member and associated with his clinic, and if the member himself did not have a paper elsewhere on the program.

In 1921, the minutes of the Council meeting contain the first mention of what many Society members consider "the most important thing in the Constitution"—the provision for emeritus membership. Council discussed the matter at that time, but the record gives no information beyond the decision that it was a subject which could most properly be presented in the form of a constitutional amendment if any member so desired. Such an amendment was introduced two years later and passed in 1924. It read as follows:

> The number of active members shall be limited to two hundred. Active members, whose membership has extended throughout 20 years, will automatically be transferred to emeritus membership, and those whose membership has extended throughout 10 years or more may at their own request be transferred by the Council to emeritus membership. Emeritus members shall be entitled to attend all meetings, but not to vote or hold office. They shall be exempt from dues.

This was modified in 1929. That year, Council heard a letter from Dr. Reginald Fitz urging that membership in the Society be increased to allow men to be elected earlier in their careers. After lengthy discussion, it was the consensus of the Council "that the Society should be kept in the hands of younger men." To effect such a policy, rather than lift the ceiling on membership, it was decided to make transfer to emeritus standing automatic at the age of 45. Dr. Walter W. Palmer was delegated to present an amendment to that effect at the business meeting and it was approved the following year. With its adoption, 55 members moved into the emeritus class.

Still another indication that the Society was rapidly splitting its original seams was the discussion, on several occasions, of the possibility of establishing subsidiary societies in order to accommodate the younger men. This matter first came up in 1921 when President Leonard G. Rowntree (28) called attention to the Society's obligation for "the diffusion of a scientific spirit." After dealing at length with the elements of this spirit—its acquisition, cultivation and dissemination—he opined that the Society could, and should, do considerably more to further it. To this end, he advocated the formation of local societies on which the Young Turks might draw for their membership and appointed a committee consisting of Drs. Miller, Peabody and Hewlett to explore the possibility. The committee's report, made the following year, follows:

> After investigation and consideration of the subject your committee believes it inadvisable at the present time to establish branch societies. In all of the larger cities there are medical organizations in which the young man in internal medicine may become a member and thus obtain the stimulation which arises from contact with others.
>
> It is recommended, however, that every member of the Society try to keep in touch with younger men of special promise in his community and encourage them to qualify for membership in this Association. By pursuing this course all eligible physicians may be given the opportunity to become members.

The idea, however, did not die. Despite the committee's optimism, there was a clear need to make provision for the rapidly rising numbers of young men eager to participate. Dr. Meltzer was among those who expressed concern over this matter, as evidenced in the following account by Dr. Pratt (8):

> I saw him (Meltzer) in New Haven a few months before his death (in 1921). He was in bed recovering from a severe attack of cardiac asthma during the preceding night. I had arranged a consultation with Wilder Tileston. When we entered the room, Dr. Meltzer refused at first to discuss his own symptoms but at once began to talk about the Society for Clinical Investiga-

tion. He said "I think the Young Turks are getting rather old. I believe there is a great need for a second society composed of younger men." Even at the time he spoke, which must have been ten years ago, conditions were similar to those that existed when he formed our society.

The need for a junior organization is still greater now (1934). Young men have to wait as long before they gain membership in the ASCI as we were compelled to thirty years ago before getting the privilege of presenting our work before the AAP.

The idea of junior societies was particularly dear to Dr. Rowntree and in 1927 he and his colleagues at the Mayo Clinic proposed the formation of the Central Society for Clinical Research. In a letter to the Council, Dr. Rowntree asked the blessings of the Young Turks for the new organization. After lengthy discussion, the Council drew up the following recommendation for presentation at the business meeting:

In view of the benefits to be derived by younger men in a society for clinical investigation, this Society is glad to learn that the organization of a society with a similar purpose is contemplated in the Central West. The Council heartily endorses this move and recommends that this Society extend its best wishes for the immediate success of the new society.

This recommendation was approved by the membership initially, but as the business meeting drew to a close, Dr. Alfred E. Cohn moved to re-open discussion and suggested that the chair appoint a committee to consider relations of the Young Turks to other societies for clinical investigation in the United States. His motion carried and a committee consisting of Drs. Homer F. Swift, Rowntree and Longcope was formed. Although the minutes note that it was dissolved two years later, no account of its findings is given. Presumably, although the Young Turks did not feel it desirable to affiliate in any formal manner with the new society, its formation, like that of the American Federation for Clinical Research in 1941, was regarded as a happy, if only a partial, solution to the membership problem. Proceedings of the Central Society's annual meetings were published in the Journal of Clinical Investigation for a good many years.

Despite such indications of growth, the Society up until the Second World War retained many of the attributes of an intimate, informal organization. Emeritus members reminisce about the "good old days" when meetings were small—say 100 or 150 in attendance—and the "boys took down their hair and tore into each other." Not the least of the benefits derived from such meetings were the friendships they fostered. In this respect, the "extracurricular activities" of the Young Turks were frequently as important as the scientific sessions, and a number of traditions evolved. It was customary, after adjournment, for members to reassemble in small groups in the hotel rooms to rehash the program—with or without the usual beverages of prohibition days. Later, many of the post-meeting discussions were held at the Brighton Hotel where members "let down the bars" over the famous Brighton punch, a rum drink remembered almost universally by men who were active in the Society during the late '30's and '40's. Since then, the Brighton has been demolished and, although its successor—the Colony Resort Motel—still has proprietary rights over the "Brighton" punch, the luster associated with it in the old days is gone.

From the Brighton, the Young Turks often proceeded to Hackney's for lobster and thence, not infrequently, back to the Brighton. In any case, it was, traditionally, "a pretty rough night." The aftereffects were equally traditional. According to one member, "No one ever came back without some kind of intestinal upset," a situation which their wives blamed on drink but which the Young Turks themselves were inclined to attribute to lobster. There was some truth in the latter interpretation for, as another member put it, "There is little doubt that Atlantic City sea food had been a long time away from the sea—it usually came from New York."

Whatever the truth may be, it should be emphasized that these annual "Monday nights" were profitable as well as enjoyable. They resulted in the formation of many close and lasting friendships and saw, no doubt, the excited conception of many ideas which were to come to life in the form of substantial contributions to the progress of medical science.

III. CONFLICT AND COMPROMISE, 1930–1945

Scientific Soul-Searching

The burgeoning of interest and activity in clinical research which had accompanied the Society's first 20 years of existence was not regarded by all investigators as an entirely unmixed blessing. By 1930, there was a fair danger that the wave of popularity on which investigative medicine seemed to be riding would turn into a veritable deluge, swamping the "boat of progress" to which Dr. Meltzer had alluded some two decades earlier and wresting it from its course. There seems, in fact, to have been some confusion as to what the true course was, a matter which received considerable attention during the Society's third decade. Dr. Loeb, president in 1936, made some particularly pungent observations on the situation (*27*):

> Keeping pace with the spirit of abandon which characterized the florid twenties, clinical investigation ran riot, recognizing no bounds, philosophical, intellectual, technical or financial. . . . Under this spell of enthusiasm it seemed reasonable to expect that with organized groups of investigators, spacious laboratories, endless equipment and technical assistance the mysteries of medical science must bow before the concerted onslaught and bow promptly. Medical students and young graduates, without consideration of "Geist" or other qualifications, were urged to enter laboratories. They might cooperate in the contemplated discovery of the etiology of cancer or they might be set the task of counting paving blocks—it was immaterial, but all in the name of science.

From many quarters, voices were being raised in concern over what Dr. Loeb termed "this great orgy of clinical investigation." Prevailing attitudes, underlying concepts, methodological convictions—all were called into question. Were the universities turning out anything more than rank after rank of highly skilled technicians? Was there any coherence amidst the feverish flurry to produce and publish; or were physicians, in their zeal to be men of science, concerned only with the fragmentary analysis of the phenomena of disease, failing in their haste to ask significant questions and ponder the meaning of their answers? Were some being motivated merely to do the "fashionable" thing? Why the deification of measurement, the aura of sanctity surrounding experimentation? Had clinical investigation been too narrowly defined? Was it losing sight of its "clinical" aspect? Wherein lay its future?

These were among the questions being debated throughout the late 1920's and '30's. Certainly some of them had been asked and answered earlier, and certainly most of them continued to be re-asked and re-answered later. But, roughly speaking, such questions seem to have come into particular prominence during the Society's third decade.

The writings of Dr. Alfred E. Cohn are illustrative of the type of re-examination of medicine's philosophical bases which was prevalent in some academic medical circles of that day. Many of his essays dealt with the question of whether clinical medicine could legitimately be considered a science, a question which was still much debated during the 1930's and which, particularly in British medical circles, was quite often answered in the negative. Cohn was an ardent apologist for the view that medicine was, indeed, a science and one of his earliest (1924) essays, "Purposes in Medical Research: An Introduction to the Journal of Clinical Investigation," was devoted to a discussion of this belief (*29*). The following excerpts from this article may serve to indicate his general approach.

After enumerating historical instances in which medicine, to its detriment, had been sidetracked by attempting to borrow the methods of other disciplines (the iatro-mathematical school of Borelli, for example) for application to the problems of human disease, Dr. Cohn said:

> The employment of a method presupposes that . . . the method selected is advantageous in the solution of the problem. . . . Mathematics, mechanics, physics, chemistry, physiology as independent disciplines has each had its proper objects of inquiry; all have been aware of their appropriate problems in the phenomenal world. . . . But the primary objects of interest in medicine cannot properly be stated in terms appropriate to them. . . . Medicine must, like the other sciences, be properly credited with having specific objects of interest on its own account. If it is true that medicine has not always been clear as to what these objects are, this may be due to the fact that the definition of its objects has not always been clear. It may perhaps be for this reason that it has so often been deflected from the straight path of its proper pursuit.

In proceeding to his own definitions of the

"legitimate scope and objects" of medicine, Dr. Cohn said:

> The phenomena of interest in medicine are the phenomena of disease as these are manifest in affected persons. They are phenomena which exist as concrete entities in nature, they are indivisible, and they fall within the province of no other inquiry. They constitute the proper concern of medicine. . . . When we come to the question of how to investigate them we find that they are to be studied by no single methodology any more than are those of the (other) . . . sciences. The methods to be employed are those which are appropriate to illuminating the specific problems in question.

The increasing tendency among investigators to adulate experimentation at the expense of the descriptive method received the frequent attention of critics during these years. It reflected a significant change then occurring in medical research: the growing reliance of clinical investigators on experimental and quantitative methods (11). The shift produced a rift of some proportions among clinicians. The experimentalists regarded representatives of the descriptive school as being somewhat old fashioned, while the latter decried tendencies to limit the definition of research to experimentation alone and, in the words of Shryock (11), to accept "any experimentation whatever as *ipso facto* worthwhile." This split affected the Society as well. By the late 1930's, most of the members of the Council were experimenters and, according to Dr. Isaac Starr (30), "looked upon the descriptive school as being dated." This fact inevitably influenced the Council's selection of candidates for membership and, as would be expected, brought numerous repercussions.

In 1931, Dr. Francis G. Blake touched on this issue during the course of his presidential address (31). Discussing the meaning of the phrase from the Society's constitution—"by the methods of the natural sciences"—he said:

> At the risk of stressing the obvious, let me make it clear at once that I conceive this phrase to refer, not to the techniques or tools used, but to the methods of approach employed in the natural sciences. There are, of course two methods—on the one hand, observation, analysis, and deduction, the so-called descriptive method, still held honorable by some because of its antiquity if for no other reason; on the other hand, the inductive, experimental method, held, it would appear, in higher esteem by most because of its relative youth and vigor.

He continued by calling argument concerning the relative superiority of either method a "somewhat fruitless pastime" and terming "narrow and partial" the view that the experimental approach was necessarily preferable. Both, he said, have their proper place. The important thing was that the investigator stop to think about what it was he hoped to accomplish, regardless of which method he chose.

The importance of clinical observation was further emphasized by Dr. John R. Paul in 1938 in an address entitled "Clinical Epidemiology" (32). He outlined what he called a "new science" in which he proposed that Society members take part by making observations on what happened to patients outside of the hospital as well as inside. Thus it was a science concerned with the circumstances, whether functional or organic, conducive to disease. But to qualify for the name "clinical," Dr. Paul said, such a science would have to concern itself with the interpretation of these circumstances, branching out from a study of the sick individual to a study of his situation, bringing critical judgment to bear on the context out of which disease had arisen. Such a concept, Dr. Paul noted, was old hat to the family doctor. But, he said, "Now that emphasis, for this Society at least, has shifted away from the home and into the Hospital and Dispensary, clinical epidemiology will be practiced only if we take thought of it."

The real value of such studies, Dr. Paul emphasized, lay not simply in the discovery of new intrinsic or extrinsic factors, but in the discovery of new concepts. He continued:

> This is all so obvious that it hardly seems worth mentioning, and yet a dominant thing about some of our present notions of causative factors is that unless they fit into a modern pattern of our own liking they are apt to be overlooked. Of late years, conservative opinion does not allow anything to be really considered as "etiology" unless we can succeed in getting it into a test tube, unless we can precipitate it—unless we can crystallize it, as it were. This is due of course to our current methodology which has, perhaps, become more of a religion than most of us realize. I think it may have led to a slightly narrow interpretation of clinical investigation on our part, for clinical investigation must be given the opportunity to spread itself up into philosophy if it will, as well as down into the basic sciences.

Although there was no serious argument with the need for a philosophical and synthetic approach to experimental medicine, the main current of development in clinical investigation continued to be analytical as new chemical and physical methods were introduced into the study of disease processes. Despite the paternal admonitions of many of the older members, the Young Turks continued to seek the "precipitates" and "crystalline substances" associated with disease. In this search they were aided by a multitude of new instruments rapidly becoming available for application to clinical problems, instruments which frequently opened up whole new areas for research (11). Medical technology grew increasingly complex. At one point, it was seriously suggested that medical schools should establish separate "departments of instrumentology" for the common use of all staff members, a development which might have avoided what Dr. Carl V. Moore called in 1954 "scintillation counter-Beckman disease" in which every department and laboratory engaged in the conspicuous consumption of apparatus to the detriment of the budget and without regard for duplication (33).

Some of the older members viewed with alarm what seemed to them a bewildering succession of "fads and fashions" in medical research. The younger men, they felt, in their anxiety to succeed in the "right" field often made idols of each new methodological innovation that came along, worshipping now "at the shrine of the colorimeter," then "at the shrine of the electrocardiogram" and later still, "at the shrine of the potassium ion." While some such cautioning was undoubtedly well-taken, the majority of the young men on the firing line felt that they were able to adopt new methods and acquire new technical skills without losing perspective on the ends which were thereby being served. The science of clinical investigation was, in the long run, greatly enriched by the growing array of instruments and techniques which enabled clinicians to transcend simple observation in their study of the mechanisms of disease.

Space, Time, and Learned Societies

Moving out of the '30's the focus of presidential concerns seemed to have undergone a slight shift. Greater emphasis was placed on what might be called functional aspects of the organization—specific policies or general considerations of purpose—and less on scientific points of view. True, the two are in some sense opposite sides of the same coin. The scientific substance which comprises the Society's "raison d'etre" is, at bottom, the factor which determines its policies and which must be kept in mind in guiding its practical affairs. But, whereas presidents during the '30's seemed to concentrate on the scientific side of the coin, those of a little later period tended to reverse it. Thus, Dr. Tinsley R. Harrison (34) dealt, in 1939, with what he called "institutional arteriosclerosis." (This address, incidentally, was never delivered before the Society. Dr. Harrison had prepared it for the meeting and submitted it to the Journal, but in a last minute burst of enthusiasm about some research which had just been done in his laboratory, he decided to tell the Young Turks about that instead. The originally-scheduled address was, however, printed in the Journal.) Noting that medical societies and medical schools, like other cultural organizations, were subject to decay, Dr. Harrison suggested that the root of the trouble, so far as intrinsic causes were concerned, was failure to select the best possible men.

In drawing attention to the importance of choosing good men, Dr. Harrison touched upon one manifestation of a more fundamental problem, one which had become increasingly troublesome as the boom in research activity brought pressure on the Society to expand its membership and scientific programs. It was a problem which Dr. Isaac Starr treated more extensively when he succeeded Dr. Harrison to the lectern in 1940: that of the conflict, common to many learned societies, arising out of the dual aims of setting a standard and performing a service (35).

Dr. Starr, according to the minutes of the 1940 meeting, "addressed the Society on the subject of functions and dysfunctions of learned societies, providing ingenious models representing important concepts of space and time in their relation to the duties of said societies." This was in reference to what must have been one of the lighter moments on that year's program, a demonstration staged by Dr. Starr with the assistance of what

was described as "a curious looking object which at a distance resembled an orange thrust through with a multitude of long steel knitting needles that radiated from it in all directions . . . placed beside a clock." It was, the president solemnly explained, "a representation of our Society radiating its beneficent influence in almost every direction. . . . This timepiece," he continued, "is an integral part of the model, for it indicates that the influence of our society extends, and will extend, throughout time." It was also, as Dr. Starr went on to announce triumphantly, a demonstration of interorganizational one-upmanship, for the addition of the clock made *his* model four dimensional and therefore one dimension up on that produced by his friend, Dr. DuBois, for the benefit of the AAP audience the preceding year (36).

Dr. Starr's visual aids, however, supplemented an otherwise serious talk in which he discussed the two functions of the Society—those of performing a service and of setting a standard. Among its services he enumerated first, the publication of the Journal which he termed a "godsend" to "those of us who are considered to be clinicians by physiologists, biochemists, and immunologists; and considered to be physiologists, biochemists, or immunologists by most clinicians." By serving as an organ for clinical research made by quantitative methods, the Journal, he said, was "in the forefront of that change from descriptive and qualitative methods to the more exact quantitative measurements . . . which has made possible the great advances of physics and chemistry and may well produce as great a revolution in medical practice."

Second among the Society's services he listed the annual meetings. Here, Dr. Starr was a little more critical. "Our programs have contained very little of what might be called synthetic thinking," he said. Insistence on demonstrated fact had been important in rescuing medicine from the "fanciful speculation" that had characterized it 100 years earlier, but, Dr. Starr stressed, all facts are not equally important:

> In every investigation there is a period when one must decide which facts are worth discovering. To do this logically, one must picture to himself what the situation is. . . . In my opinion, our program would be improved by studying the methods of the great English school of physiologists whose members have never been afraid to attempt to synthesize the facts into simplifying theories. . . . We are spending much time, effort, and money in our clinics accumulating records, apparently in the pious hope that some day somebody is going to synthesize something useful from the multitude of data they contain.

The Society's third means of performing a service was considerably less tangible, Dr. Starr said, but no less important. It consisted of fostering acquaintance among investigators. Often, he felt, personal questioning revealed far more about a subject than did reading of the literature, and he expressed the hope that contacts afforded by Society meetings would be of the sort to facilitate cooperation rather than to create rivalry.

By and large, Dr. Starr thought, there was little doubt about the quality of the services rendered by the Society. The real problems began when it attempted to exercise standards through such functions as the selection of members and of papers for the scientific program and the Journal. In all cases, the pool from which selections had to be made was large in comparison with the number which could be chosen; the task of selecting was complicated and mistakes inevitable. This underscored his final point—that the dual functions he had outlined were to some extent conflicting:

> There are some who think that the membership should be enlarged and the program expanded. Their aim is to improve the service given by the Society. This is opposed by others who point out that such a change would lower our standard of excellence.
>
> Too much emphasis on standards is a cause of decay; often it is a psychological defense mechanism set up by persons no longer productive. The organizations which become more and more exclusive tend to die of dry rot. Mistakes made in the enforcement of the standard make them ludicrous. . . . Nevertheless, while undue insistence on standards causes difficulties, abandoning them too far in the interest of service brings troubles of another sort in its train. For then the door is opened for the man whose real aim is personal advertisement rather than the search for truth. Uncensored programs are likely to be too long. Societies with low entrance requirements become so large that friendship between the members becomes impossible and the personal service on which I lay so much value is non-existent.

Thus, Dr. Starr continued, a compromise between services and standards was necessary and in attacking this problem, as well as others, he felt that two principles should be kept in mind. Dramatically, then, he produced his model. In all its

four-dimensional glory it was intended, he said, to illustrate his first point:

> In dealing with learned societies, let us preserve our sense of humor. In some of the letters received by the secretary a sense of humor has seemed strangely lacking. The errors of today, if they be errors, can and must be corrected tomorrow.

He concluded by urging as a second general principle the greater use of ordinary processes of democracy. All too common among medical societies, he said, was the tendency of members to let officers handle all the affairs and to be negligent in attendance at business meetings. Against this tendency he cautioned the Young Turks to be on their guard.

Dr. Starr's message is an important key to an understanding of the basic issues at stake in the debates and decisions which constitute the Society's policy-making history. From the outset, the Young Turks had stood for quality in clinical investigation but, while it was never suggested that the traditionally high standards should be sacrificed, the problems of maintaining them in the face of recurrent pleas for enlarging the membership and expanding the program—pleas, essentially, for an extension of services—grew more and more difficult. As Dr. Starr pointed out more recently (30):

> While I was a member of the Council (1939-40) the problem of policy being vigorously debated was one much to the front throughout all American learned societies. Of the societies senior to ours, the Association of American Physicians had taken one course; that is, it had continued a policy of limited membership and still has maintained that tradition, and (has) continued the tradition, of one program. On the other hand, the societies of the Federation, like the Physiological Society, followed the course of admitting everybody who seemed to be qualified, allowing every member to present a paper, and simply expanding the program until there was a multiplicity of simultaneous sessions.

For the Society, the decision was not quite so clear-cut. Debate over membership policy and type of program had been a continuing phenomenon since Dr. Meltzer's fervent plea at the 1914 meeting, but during the 1930's, the problems of accommodating more men and more papers became particularly acute.

The Young Turks had entered the '30's with their collective heads bumping against the 200-member ceiling which had been imposed in 1924—the 1929 catalogue lists 201 active, and 34 emeritus, members. When, in the following year, transfer to emeritus standing became automatic at age 45, some head room was provided, but the repreive was only temporary. The number of candidates tended upward while the number of available places fluctuated but little and, by 1938, membership in the active category had climbed back to 199 and emeritus members numbered 174. Thus, within 10 years the Society had once again reached its upper limit and as it emerged into the succeeding decade it had, literally, to raise the roof. Constitutional amendments introduced in 1941 abolished the limit of 200 and established, instead, a quota on the number to be admitted annually—either 25 or the number corresponding to the number of transfers to emeritus status, whichever was larger. Although these changes effected a slightly more flexible membership policy, they did not forbode a significant increase in the Society's size, and it was in this year that Dr. Christian led a number of the disgruntled candidates off in search of a new society, the American Federation for Clinical Research.

Not only the size, but the composition of the membership was called into question. We have already noted that the experimentalists were at odds with representatives of the older descriptive school. There was, in addition, some debate arising out of the increasing degree of specialization in clinical research and its effect on the selection of members and programs. According to Dr. William S. Dock (37), an active member throughout the 1930's and early 1940's, "The row over making us all good internists, equally interested in physiology, biochemistry, microbiology, endocrinology and hematology or specialists listening only to other specialists began during this period." In later years as well, members have alternately petitioned Council to restrict membership to internists or to counterbalance their preponderance by admitting larger numbers representing other clinical specialties or the basic sciences. Pressure has been exerted, too, for sectional meetings for the presentation of papers dealing with subdivisions of clinical research, but not until very recently has this suggestion been effected through the joint Society-Federation programs.

Problems have arisen, too, in connection with

the criteria to be used by the Council in the selection of members. There have been instances in which men of recognized merit as teachers present but limited evidence of productivity as investigators. Often, these are men of outstanding ability, highly esteemed by their colleagues in academic circles, and it is a ticklish matter to exclude them from membership on the basis of their limited research activities. Another delicate consideration sometimes arises in connection with the academic affiliation of a nominee. There is an inevitable tendency to regard a candidate from an outstanding institution with greater favor than a candidate from a less well known school, primarily because the standards against which the former has been measured and the men with whom he has worked are a known quantity to the members of the Council. On the other hand, there have been vigorous debates over nominees from lesser institutions who, although sometimes less qualified on an "absolute" scale, have made significant achievements relative to the conditions under which they have worked. Decisions in such cases involve the more fundamental problem of how the Society can best promote "the cultivation of clinical research"— whether by recognizing the prior attainment of high standards or, in some cases, by fanning a spark of interest and ability in research existing in the "outlying regions" where less favorable circumstances prevail.

The question of expanding the program beyond the traditional single session, single day limits has been one of the most vigorously contested issues in the Society's history. In 1921, a two day session was considered and rejected; in 1927, the division of the meeting into three sections was considered and rejected; and in 1932, an evening session was considered and rejected. Thereafter, the issue raised its troublesome head with almost annual regularity. No sooner was it disposed of at one meeting than it would be reintroduced at the next and a new proposal for its solution subjected to a repeated vote. Probably no other problem has received such persistent attention from the membership at large—it seems to be one of the few issues which was handled as often on the floor of the business meetings or through the mails as it was in the sessions of the Council.

The advocates of change won out for the first time in 1934 when approval was given for a trial departure from tradition for the following year. Thus, the twenty-seventh annual meeting of 1935 was divided into two simultaneous scientific sessions with 22 papers scheduled for presentation in each. The success of this innovation was discussed at a general business meeting following the completion of the double program and a variation adopted for the following year. The 1936 meeting, thus, consisted of a general morning session and a double afternoon meeting. This plan, actually, provided no solution to the problem of an oversupply of papers for only nine were scheduled for each of the three half day sessions and the total of 27 thus presented was no greater than that normally appearing on single session programs.

After these two years of experimentation, however, the Society hastened to retreat. In 1936, "after mature consideration of the question," it was the opinion of the Council that "the interests of the Society could best be served by a return to the single session program." What these "best interests" were is summed up in the following views expressed by Dr. Paul (38):

> In retrospect, at least, I believe that the Society's strength was enhanced by the fact that it took upon itself the task each year to pick the best from a long list of titles and to condense their presentation into a single day. This was not the practice of other societies which went in for quantity rather than quality. It meant that sometimes about four times as many titles were submitted to the Society as were actually presented and loud was the complaint from some that good material was not being heard. However, in the long run this seemed to react favorably and it never seemed to deter the membership from submitting its long list of titles. It also served to raise the stature of the meetings. To be chosen as a speaker at the American Society for Clinical Investigation has become an honor of growing importance.

Although it was such considerations as these which led to the 1936 Council decision, a final settlement to the problem had not been achieved. Criticism of the selection of papers was sometimes quite vehement, presidents being accused, on occasion, of biasing the programs in the direction of their own scientific interests or of taking considerations other than merit alone into account. Dr. Paul recalled "a storm of protest" one year

when the president announced that he had leaned heavily on the geographical distribution of authors in making his choices. Dr. John P. Peters, whose voice was often raised and usually respected in the Society's decisions on policy was especially vociferous in disapproving this particular innovation. Thus, the debate went on. In 1939, a new Council voted to resubmit the type of meeting question to the Society, urging by a five to one margin that the double session program be reinstated. Opinion among the general membership, however, was more evenly divided. At the general business meeting, the Society voted to retain the status quo although the double session plan lost by only seven votes (47–40). In 1940, the Council again took up the question, this time in response to a letter from Dr. Peters urging reconsideration. The matter was resolved by conducting a post card ballot, among active members only, in time to permit decision as to the form of the 1941 meeting. A similar referendum was held prior to the 1942 meeting and on both occasions a plurality favored a half or whole day double program at the discretion of the president after inspection of the quality of the abstracts submitted. With this mandate from the membership, Presidents William B. Castle (1941) and William S. Dock (1942) scheduled double morning and single afternoon sessions at which, in each year, a total of 45 papers was presented.

World War II twice disrupted the orderly progression of annual meetings—in 1943 and 1945. In response to a memorandum from the Office of Defense Transportation requesting the cancellation of meetings during 1943, President Eugene M. Landis wrote for clarification of the Society's status, outlining the nature of its meeting and the expected attendance. A reply from the Director of the Division of Traffic Movement indicated that the final decision was up to individual organizations themselves, but that unless the meeting had a direct bearing on the war effort, its omission would be greatly appreciated in light of the need to alleviate the burden on transportation facilities. The Council members were unanimous in their decision to cancel the 1943 sessions. Similar considerations applied in 1945. In both years, Council members met in Cleveland, Ohio, to carry out such routine functions as the nomination of officers and new members and to transact other necessary business. Decisions, along with the minutes of the meetings, were transmitted to the membership for approval by mail.

These war years were thus a kind of interlude in which little significant action could be taken and in which pressing problems were temporarily shelved.

IV. THE TEMPERING OF TRADITIONS, 1946–1959

If clinical research activity had boomed in the 1920's and 1930's, it exploded in the years following World War II. In the interval between 1945 and 1955 alone, the number of physicians trained in clinical investigation increased tenfold. The funds available for medical research likewise multiplied at a rapid rate, in large part due to the entry of the federal government into the support of scientific activities on a massive scale. According to Dr. W. Barry Wood, Jr., president of the Society in 1952, there are some who believe that the postwar growth of clinical investigation has actually been too great in proportion to the growth of research in the basic sciences (although the latter has also increased tremendously). This point of view stands in striking contrast to that professed by Dr. Meltzer in 1909 and serves to indicate the amazing changes which have taken place in the status of his "new science."

The effects of such changes on the Society's already swollen membership ranks need not be elaborated. Suffice it to say that when the Atlantic City sessions were resumed in 1946, many of the same internal and external tensions were still present, but in the postwar years they have taken on new and complicating aspects. Not only, for example, did the Society have to decide what to do about a mounting number of nominees. In addition, it had to decide what to do about an unprecedented number of spectators. The fundamental conflict between high standards and more extensive services manifested itself anew in such bitterly-disputed decisions as the move to the Steel Pier Theater and in the continuing debates over membership and programming policies. In some matters, the decisions of an earlier day were reaffirmed. In others, it was necessary to modify traditional policies in accordance with the demands of changing times.

After lying dormant for a number of years, the "type of meeting" question began to reappear on Council agendas after 1949. No longer, however, was the central issue one of single versus double session programs. Rather, discussions revolved about the possibility of extending the program to more than one day and of doing so in cooperation with the "senior" and "junior" organizations, the Association of American Physicians and the American Federation for Clinical Research.

It is interesting to note that in 1939 and 1942 Society members had rejected firmly the third alternative of a two day meeting. It was this alternative, however, which received major consideration in the years following World War II. Thus, in 1949, in view of the Society's traditionally close relationship with the Association of American Physicians, the Council instructed the incoming president to confer with the incoming president of the Association regarding the possible integration of the two programs. If such consultation actually took place it apparently came to nothing and is not mentioned subsequently in either the Society's records or the Transactions of the Association. Three years later, however, agreement was obtained on a proposal to extend the meeting to a day and a half, provided "suitable arrangements could be made with the Association." That organization's Council agreed to the change under which the Association would have met all day Monday and on Tuesday morning; the Society, Tuesday afternoon and all day Wednesday. Fortunately or unfortunately, however, the plan never got beyond a tentative stage. When it was discovered that the Haddon Hall Hotel could not accommodate Society members on Wednesday night, the traditional single day meeting was restored to the traditional Monday where it has remained ever since.

Two years ago, in 1957, a special committee consisting of Drs. R. V. Ebert, S. J. Farber, J. D. Myers (chairman) and L. A. Rantz was appointed to consider once again the form of the annual meeting, and this time a major change was effected. According to its report, "The committee decided that the time had come when there should be sectional meetings in our program, that is, meetings of appropriate sections of the general field of clinical investigation in medicine." These meetings were to be in addition to the usual general session and were to begin in 1958. They were to be held on the Sunday afternoon preceding the Society's regular session, thus running concurrently with the regular meeting of the American Federation for Clinical Research. The committee met with representatives of the Federation and invited that organization to join with the Society

in sponsoring the sectional sessions, although it was made clear that the Society planned to undertake the project alone if necessary. The invitation to joint sponsorship was accepted by the Federation, details were worked out by the presidents of the two organizations, and in 1958 the cooperative sectional meeting plan was inaugurated. Repeated in 1959, the sections are no doubt here to stay and represent, it would appear, a partial solution to the long-standing problem of accommodating a greater number of papers and a happy compromise in the controversy over a specialized versus general interest program.

Although the question of increasing membership was aired by the Council in 1950 and 1951, stimulated on both occasions by letters from Society members urging expansion, no changes were made until 1952. In that year, the Society adopted three amendments which *1*) increased the annual quota on new members to 35 (previously 25) or the number equal to the number of retiring active members, whichever was larger; *2*) allowed members to request transfer to emeritus standing after five, rather than 10, years of active membership; and *3*) made the nonpayment of dues for two, rather than three, successive years a cause for forfeiture of membership. To date, these are the last changes which have been made in membership policy. Thus, although the Society has made provision for slight increases in size, the guiding principle has remained that of keeping the organization small and highly selective. Addressing the Society in 1957, President Stanley E. Bradley presented an eloquent argument in support of this position (39). Tracing the evolution of "the tradition of scientific critique" through the 300 year history of learned societies which had been "formed to defend and exploit the new (scientific) method," he discussed in particular the Royal Society of London, showing how its influence had waxed and waned as it had alternated between maintaining standards of the highest excellence, allowing them to fall, and again restoring them to a high level. Then he continued:

> The parallels between the story of the Royal Society and our own need not be belabored. We, too, are faced with the problems posed by an unsettled membership policy, by an ill-defined relationship to other groups, and by pressure for expansion. . . . Our Society also has stood for quality, for the highest possible standards of excellence in membership, in the Journal and in the papers presented before this meeting. . . . No standard that lacks divine sanction is readily acceptable; jealousies, resentments, fears, a host of varied considerations combine to raise up an active opposition to it. . . . Criticism of the Society is especially acrid in connection with the choice of new members. In eight years of work on the Council . . . I can say with pride that I have never seen any consideration other than merit take first place though geographical distribution is often urged upon us. . . . More recently there has been pressure to increase the membership on the assumption that the number of new members should be determined by the number of men nominated. The history of the Royal Society shows the fallacy and danger of this assumption. If we wish to allow for the growth of the research establishment in this country, we might gear membership to the number of workers listed in American Men of Science, or better yet, to the number of subscribers or contributors to the Journal of Clinical Investigation. Certainly the number nominated is a poor criterion of anything but the prestige of the Society.

The "prestige of the Society" gave rise to another problem in addition to the growing number of nominees—the problem of the growing number of nonmembers attending the annual meetings. Acting on the basis of letters from a number of members urging that a larger meeting hall be found, the Council, in 1950, initiated what is quite possibly the most radical change in the entire history of the Society—the move from the relatively small chambers of the Traymore and Haddon Hall Hotels to the Atlantic City Steel Pier Theater, a huge auditorium. The move was recommended on a trial basis for 1951 and a short Council meeting following that year's experimental sessions resulted in a decision to return in 1952, a decision which has been followed ever since.

The official records of the Society contain only two references to the change—the initial decision is set down in a three sentence paragraph in the minutes of the 1950 meeting of the Council and two additional sentences in the 1951 records suffice to indicate that the change was to be permanent. Despite this secretarial brevity, however, such a move had been under consideration for some time prior to 1950 (although probably not before the end of the war) and was not greeted with anything resembling the universal docility which the records alone might lead us to surmise. Rather, in the words of Dr. Wood, who served on the Council between 1947 and 1949, "The move to the

Steel Pier was made reluctantly, only after the pressure of the audience made it necessary." The decision, when it finally came, Dr. Wood pointed out, was the outcome of a struggle between proponents of two opposing views: those eager to maintain the type of meeting in which informal discussion was possible, and those who urged that their "inspiration value" be extended to a wider audience. In the end, the latter view won out but, according to Dr. Wood, "it took quite awhile for this to break through."

Traditionally, although both members and guests had increased in number, the Society had adhered to the view that its meetings were the occasion not simply for the presentation, but for the exchange, of ideas relating to scientific work. True, time for discussions had undergone considerable trimming since the early days. But the prevailing atmospheric conditions at meetings had been conducive to easy and informal give-and-take. Although intimacy was relative, it had been fostered by the closer quarters of such meeting places as the Viking and Vernon Rooms of Haddon Hall. The migration to the Steel Pier reflected the fact that these earlier meeting places had been outgrown, but it also symbolized a new conception of what the annual meetings should represent. The attractiveness of the Society's meetings carried with it a degree of "noblesse oblige," and many members began to express the opinion that the organization had a direct responsibility, not just to its members, but to the large audiences and particularly to the medical students and house officers who made the annual pilgrimage to Atlantic City and derived from the meetings renewed inspiration for their own work. One of the stated objects of the Society, as set forth in the constitution, was, and remains, ". . . the diffusion of a scientific spirit among its members." In this connection, it is interesting to note that at the charter meeting in 1908, Dr. Theodore C. Janeway had moved to delete the phrase "among its members" from the original draft. His motion had failed for lack of a second, but perhaps in 1950 the Young Turks came back, in deed if not in word, to what Dr. Janeway had had in mind. One of their objects, thenceforth, was to be ". . . the diffusion of a scientific spirit" beyond the limits of the membership.

Be that as it may, opinion on the wisdom of the Steel Pier move remains somewhat divided, although most members seem to have accepted it fatalistically, if not exactly enthusiastically, as "inevitable." There seems to be general agreement that present day meetings are "different" but there are, on the one hand, those who say that the move has "spoiled the meetings" and, on the other, those who say it has merely "changed" them. Many who are most vociferous in their denunciation of the change end their diatribe against the Steel Pier with the concession that it does have the advantage of allowing the youthful audience—"those who need it most"—to hear the results of "the best work done each year in clinical research." Whether this advantage negates much else that was formerly of value in Society meetings seems still to be an open question.

Older members, who look back to the days when discussions were "hot and good," bemoan the loss of intimacy wrought by the change. Back in the '20's and '30's, they say, the Society was "like a family group" in which relations were friendly but no one hesitated to say what he thought.

"Now that they've moved to that Steel Pier," one former president lamented, "no one has nerve enough to get up and give the speaker hell. After all, you can't call a member of your family a name in front of thousands of people!"

Another emeritus member, also a past president, was quoted by President Robert H. Williams (40) at the 1955 meeting as follows: ". . . The program and the Steel Pier are terrible," said he. "I do not see why these meetings should be the occasion for carnivals, in which nonmembers are ten times as numerous as members."

But there are ex-presidents lined up on the other side as well. "Discussion is certainly less informal," one of them admitted readily. But, noting that "the Steel Pier is very different psychologically," he questioned whether speaking before a large, impersonal audience where one is hardly aware of anything but a vast sea of faces is really more difficult than speaking before an intimate—and critical—group of personal friends. Another ventured the opinion that discussion varied more from year to year, and always had,

than as a function of such external factors as the size of the audience or the meeting hall.

Dr. Williams' 1955 survey (he had conducted an informal poll among active members regarding their opinions on a variety of Society policies) (40) revealed some further facts in the controversy. Twenty-nine members (of approximately 100 who had replied to his letter) indicated complete satisfaction with the programs. On the other hand, 47—nearly half—of the replies he had received contained the complaint that discussion was inadequate. This shortcoming was attributed, among other reasons, to the size of the crowd and the embarrassment associated with speaking before it.[7] Not only the scientific program, but the type of personal contact which had characterized Young Turk gatherings of an earlier day had suffered in the opinion of many. Typical comments read by Dr. Williams (40) were these:

> . . . As the crowds at Atlantic City have become so very vast, it has become difficult to meet anyone except by appointment, and often then, only for brief periods of time.

Or:

> I visualize (the Society) originally as being a rather small club which had social function and a high scientific standard . . . It is no longer a social club in any sense of the word. . . . It is with the greatest difficulty that I am able to remember who is a member and who is not.

And thus, the controversy continues. Despite opinion as to the merits of the Society's newest meeting hall, it does seem in many ways to represent a satisfactory compromise in the old conflict of standards versus services. For if, as seems apparent, the Young Turks have decided against any significant easing of membership restrictions, they appear to have found in the larger capacity of the Steel Pier Theater a means of extending their services to nonmembers without sacrificing the high standards required for election.

Preliminary rumblings of pressure for another kind of move were heard in 1954 when the Council considered the possibility of meeting in a location other than Atlantic City. In a statement prepared for presentation at the business meeting, the Council members pointed out that "the practice of holding every meeting in the same city involves certain disadvantages and inequities to a large segment of our membership and guests" and recommended that a move be given "serious consideration." In his presidential address that year, Dr. Carl V. Moore included a similar plea (33):

> The time has come, it seems to me, for us to recognize the "American" in our name, to meet in other sections of the country as well as here. . . . so that the splendid influence of these sessions can be spread over a broader section of the country. Men have discussed such a plan on the Boardwalk for years; councils have debated it; the time is ripe for one of your presidents to recommend it from here.

Dr. J. D. Myers was appointed to represent the Society in consultation with representatives of the Association of American Physicians and the American Federation for Clinical Research regarding the feasibility of such a move. This committee's conclusion, reported in 1955, was that "for the forseeable future" it was advisable to remain in Atlantic City. The Council minutes state, however, that "the Society will continue *active* exploration of the possibility of meeting occasionally in a city other than Atlantic City." There is no official indication that this intention has been carried out and, to date, neither the Society nor its comrade organizations have made any overt move in the direction of another city. However, the 1954–55 consideration of such a possibility reflects certain changes in the modern day makeup of American investigative medicine and their relation to the Society. With the postwar expansion of research activity, numerous new organizations representing various subspecialties of the field have likewise sprouted. As a result, *the* Atlantic City meetings, which formerly were considered the high point of the year by clinical investigators, have become, as Dr. Wood put it, "somewhat diluted." Their quality has not been impaired, but against the background of a year-round succession of meetings taking place all across the nation the Atlantic City sessions no longer stand out so distinctly. Dr. Wood pointed out, furthermore, that the geographical center of research activity seems to be shifting away from

[7] Other reasons given for dissatisfaction with meetings were as follows: Talks too complex or technical, 21 members; talks not clinical enough, 12; 10 minutes too short, 21; a one-day program too short, 13. Correspondingly, such reasons as inability to understand talks and pressure of time were also cited as factors contributing to inadequate discussion.

the Eastern seaboard. The Society's composition from a geographical point of view was illustrated by a diagram presented by Dr. Moore (33) in connection with his 1954 address. Dividing the nation vertically into three sectors, he gave the following figures on the distribution of members as of 1954:

	Active	*Emeritus*
East Coast	176	249
Midwest	62	107
Far West	30	29

Although the 1955 decision to stay in Atlantic City was based on the fact that it was still the most convenient location for the majority of the members of the three associations, its disadvantages are becoming increasingly apparent and may portend departure from still another time-honored tradition in the future.

Another of the Society's traditional positions coming up for re-examination after the war was its attitude toward medico-political problems. Perhaps the most striking change in the overall picture of clinical investigation in this country since about 1940 has been the growth of federal support for research activities. With governmental agencies offering plums for distribution, there has been a markedly increased tendency on the part of many national medical organizations to keep a watchful eye on the federal hand that feeds them. Although the Society has usually stuck to its traditional scientific preoccupations, it has been forced, increasingly, to consider its proper attitude toward certain political questions.

Except for two resolutions which, as previously noted, were adopted in 1917, the Society as an organization had followed a policy of "nonintervention" in the politics of research, preferring to stick to the substance instead. The general feeling seemed to be that there were more appropriate channels than the Society through which individual members could act regarding matters of national policy and that nothing should be allowed to detract from the essential purpose of the annual meetings—the consideration of scientific work. This viewpoint is illustrated by a Council decision made in 1938. In that year, communications from a number of groups and individuals requesting permission to submit various resolutions to the Society had been received. The exact nature of these resolutions is not on record, but permission to present them was denied, not on the basis of their content, but because "in view of the very limited time for our meetings, the consideration of such subjects by the Society as a whole (is) impractical, and . . . any recommendation on these matters (is) outside the province of Council."

Such questions did not come up again until after the war. In 1950, Dr. John Dingle, delegate to the National Research Council, met with the Society's Council to discuss the advisability of the Society's acting as a body in expressing views on such subjects as federal support of research. Correspondence between Dr. Dingle and Dr. Thomas Hale Ham, then president of the Society, had been circulated among the members and some 75 letters received in response. After discussion, the Council concluded that many of the political issues at stake could be handled most effectively through the Society's National Research Council delegate and, to assure a closer working relationship, it was recommended that future delegates sit with the Council and present an annual report for Council's consideration. However, the minutes read, "Beyond this, it was not thought advisable to make any major change in policy of the Society. Individual members can act through other societies, their own institutions, or boards and committees on which they serve."

Similar considerations prevailed in 1955 when Delegate Thomas Hunter's report included a request from the National Research Council that the Society support a National Research Fellowship. The records state that after some discussion, "Council concluded regretfully that the Society was not in a financial position to support such a fellowship, but it was felt that the Society through its membership was providing considerable support and guidance to the research activities in which the NRC is interested."

On both of these occasions, the issue of taking stands on political policy questions was intertwined with the somewhat more diffuse issue of the Society's relationship to the National Research Council. This organization's membership consisted of representatives of "constituent societies," members-at-large, and members of the National Academy of Sciences. Since 1917, the Society

had maintained a representative, but his attendance at the annual meetings of the NRC's Division of Medical Sciences had apparently amounted to little more than a formality. In 1948, however, with the NRC coming into greater prominence because of its responsibility in handling federal funds, the whole question of the rather ambiguous NRC-constituent society relationship came up for reappraisal. A large share of the credit for the initiation of this reappraisal apparently goes to Society Delegate Dingle, but his efforts were to little avail. Attempts to work out a more precise definition of the role of member societies and their delegates soon bogged down at the various levels of NRC bureaucracy and the Society's rather exasperated attitude toward the whole issue was summed up in 1951 by Delegate Walsh McDermott in his report to the (Society's) Council:

> Under the present system, the Society representative is in effect no more than an emblem on display at the (National Research Council's) annual meeting to indicate the good faith of the Society. Accordingly, two possible courses of action could be considered: (1) continue the presentation of the Society representative as an emblem . . . on the assumption that at some future date the role of constituent societies will actually be redefined or (2) have the ASCI representation handled by one of the numerous members of the Society who would inevitably be members of the executive committee of the Division of Medical Sciences. . . . The additional effort necessary for a member of the executive committee to serve also as a Society representative would be trivial.

The Society's delegate, as of 1959, is still an "emblem" and clarification of his role still wanting.

Although Society officialdom remained firm in its traditional "hands off" policy where matters of state were concerned, there was one occasion on which the membership rose up to petition the government. In 1954, Drs. T. S. Danowski and Eugene A. Stead moved at the Society's business meeting to circulate a statement for signatures. Critical of a United States Public Health Service policy on the allocation of research funds, the petition read as follows:

> We, the undersigned members of the American Society for Clinical Investigation, are greatly perturbed by your recent announcement that competent investigators have been deprived of United States Public Health Service research funds because of allegedly derogatory information in their files. We recognize the necessity for security clearance of individuals engaged in classified research. However, it is our firm belief that awarding grants for unclassified research on any basis other than the value of the project and the competence of the investigator endangers not only scientific freedom but also the basic foundations of our way of life.

The petition was, of course, a part of the general cry of consternation being raised at that time over the "un-American activities" of Senator Joseph McCarthy and his followers but Society members had, in addition, a more personal motive for their action. Government "punishment" of individuals on the grounds of alleged disloyalty had struck close to home in the case of several Society members—most prominently, in the case of Dr. John P. Peters. In addition to being denied the use of federal research funds, Dr. Peters was dismissed from membership on a Study Section of the National Institutes of Health's Division of Research Grants and Fellowships on anonymous and undisclosed charges of disloyalty (41). According to close friends and associates, the charges were completely unfounded and Dr. Peters could have accepted his dismissal quietly. His membership on the Study Section was due to expire shortly (he had already attended the last meeting of his term at the time of his dismissal). But he chose, instead, to dispute the Loyalty Board's decision. His case was carried to the United States Supreme Court and, although he was cleared personally, the Court refused to rule on the principle which Dr. Peters had hoped to establish: the unconstitutionality of loyalty proceedings in which the accused was not allowed to confront and cross-examine the witnesses against him.

Thus, it was in Dr. Peters' defense that Society members arose in affixing their signatures to the 1954 petition and it was one of his students, Dr. Danowski, who led the fight. The motion to circulate the statement was approved without dissent and, at the meeting the following year, it was reported that a majority of the active members had approved it. It had subsequently been submitted to the Department of Health, Education and Welfare. Replies from Department officials expressed appreciation to members of the Society for making known their opinion and advised them that the "problem was under continued study."

Federal activity, as might be expected, has

prompted commentary by Society presidents. In an address entitled "Biological Beachheads" delivered at the first postwar meeting, in 1946, Dr. Thomas Francis, Jr., expressed concern over the possible effects of various legislative proposals for the mobilization of medical research forces—fear that coordinated study projects organized along the lines of the atomic bomb research groups would stifle original thinking; that any federal research agency, by the very nature of the administrative principles of a large organization, would tend to be "founded on orthodoxy" and to breed "conformity with accepted opinion" (42). Fears such as these were prevalent among scientists in the immediate aftermath of the war, but in a sense they were really old concerns under a new guise. It is interesting to recall, for example, Dr. Loeb's denunciation of the "research project" in 1936 (27) and to note that similar fears—particularly as regards the "strings" which might be attached to funds—were expressed with regard to private sources of support when these had come into prominence at a somewhat earlier date (11).

One facet of federal support which sometimes poses peculiar problems is that of Congressional preference for research leading to tangible progress on the problems of specific diseases. This has resulted in the channelling of a large share of public funds into "categorical" research, a point on which Dr. A. McGehee Harvey commented in his 1956 presidential address (43). Dealing with the role of the university medical centers in the training of investigators, Dr. Harvey traced some of the academic shortcomings to the financial straits in which many departments found themselves. One major difficulty, he pointed out, was that of the funds available too many were earmarked for specific research and could not be used for general operating expenses, the point at which many of the university departments were running short. To remedy the situation, Dr. Harvey made this suggestion:

> The Federal Government might adopt in the future an overall policy of more realistic reimbursement of overhead expense to grantees such as it does to contractees of Federal research funds. A greater percentage of the Federal money for general support of research and research training in University centers and less of the total for categorized research is highly desirable.

On other subjects as well, postwar presidents have continued to fill the role of perceptive observer or unsparing critic. Since 1946, the topics to which they have addressed themselves have been diverse, ranging from the lessons of personal experience to problems and opportunities in medical academia, from Society policies to the contemporary status of clinical research. The specific problems of today add unique dimensions, but the general areas of concern which drew comment from an earlier generation of Society presidents receive renewed consideration from their present day counterparts.

It is this same combination of continuity and change which has characterized the evolution of the Society to the present day. With respect to its internal affairs, tradition, as we have seen, has been tempered in the ever-tended fires of controversy and, although changes have seldom been radical, the policies inherited from the past have undergone gradual modification in response to the needs of the present. Less responsive than some would have it, more so than others, the Society has evolved its policies through a continual restriking of the balance. The prestige which it enjoys today can be attributed in large part to the long-standing tradition of high standards which it has maintained, at times in the face of vigorous assaults from within and without. At the same time, present generation Young Turks have not allowed their hands to be tied by uncompromising adherence to policies established by their predecessors. Through such measures as the extension of its program in cooperation with the American Federation for Clinical Research and the expansion of its meeting capacity, the Society has demonstrated a necessary flexibility. Though the wisdom of these moves may be disputed, they attest to the continuing round of conflict and compromise which seems essential to the future health of any organization.

In a broader sense, however, the history of the Society is constituted not in the evolution of policies but in the overall development of clinical investigation which has accompanied, and interacted with, its growth. The real measure of its success lies in the extent to which the objects laid down by its founders have been advanced. In this respect, the Society has a proud past. In 1957,

50 years after the Society was born in the minds of a small group of men on the Boardwalk, Dr. Bradley (39) presented the following testimony to the achievements which had taken place during the course of its lifetime:

To the best of my knowledge, no conscious plan was ever laid down to attain our Constitutional objectives, but the size and interest of the audiences we are privileged to entertain each year suggest that the Society may have been successful in spite of itself. Medical education has undergone an extensive revision in the years since the first meeting and there is good reason to believe that many members played a major role in the process. In time the triumph of the ideals to which these men subscribed gave lustre to the Society. The standards they established in selecting members reflected great credit upon and gave distinction to the fact of membership itself. The Journal benefitted as a result of the conspicuous quality of the papers it was able to attract. The conscious emulation of the accomplishments of the founding members has done much to promote "the cultivation of clinical research by the methods of the natural sciences" throughout this country and to encourage "the diffusion of a scientific spirit."

Of even greater importance has been the change in the practice of medicine during these years. There is little doubt that now science and medicine are closely blended, a result born of dire and urgent necessity. In no other group of professional workers is the scientific attitude more vital or more meaningfully a matter of life and death. Practice today is truly clinical investigation, demanding at once a vigilant critique, a full understanding of the human situation and a knowledge of fundamental scientific techniques. As it strives to assist in maintaining a high standard of excellence in clinical research, the Society fully serves the ends for which it was founded.

It would seem most fitting to conclude the story of the Society's first half century by turning toward its second with the words of this year's president, Dr. John Luetscher (44), spoken on the fiftieth anniversary of the Society's first annual meeting:

If the Society is to progress toward the promise and goals of its founders, their lively pioneer spirit must neither be drowned in excessive numbers nor immobilized in outdated tradition. Let us look to the next 50 years with a welcome to many more of Dr. Meltzer's "brainy young men" and to their fresh ideas and new programs for this ever youthful Society.

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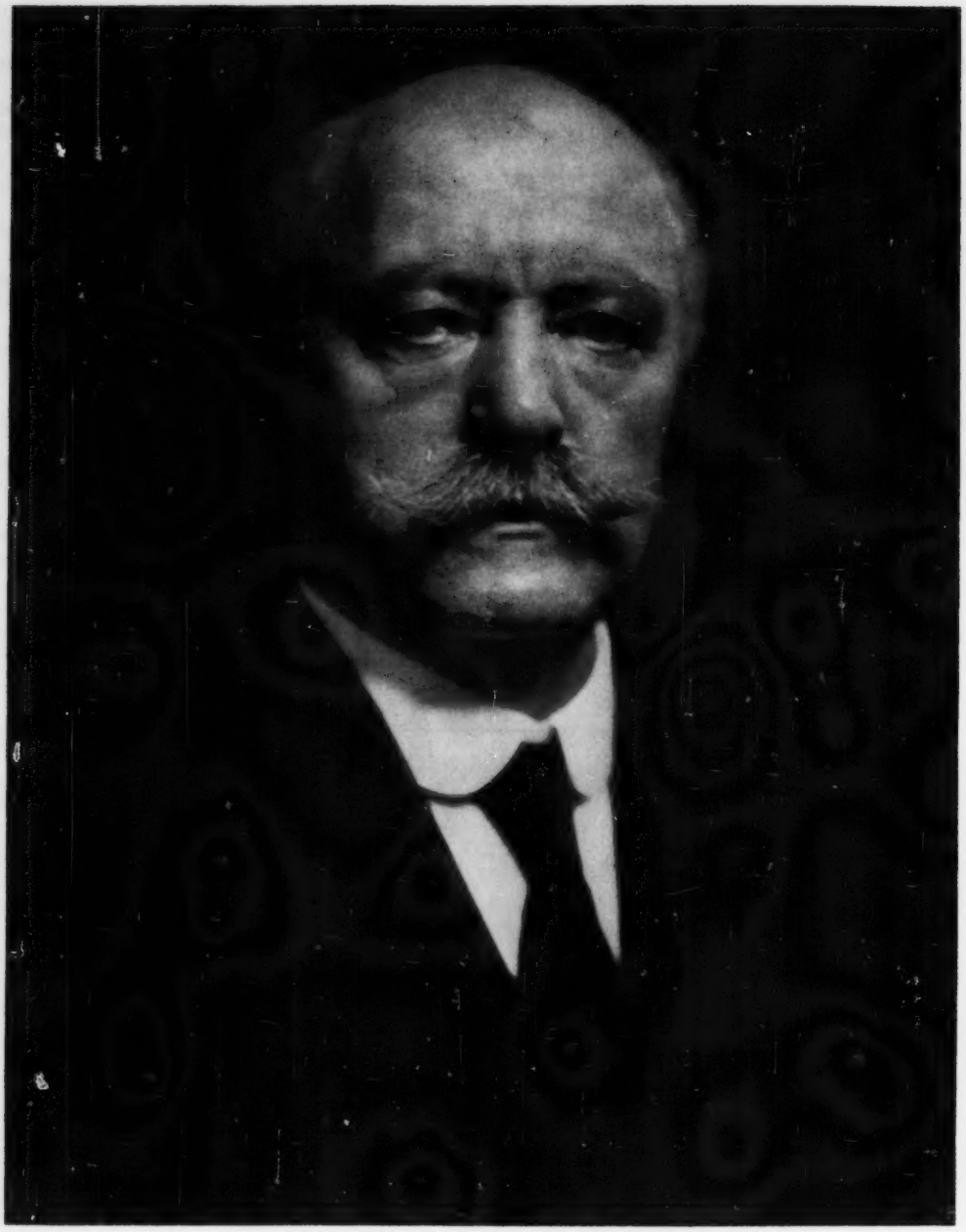
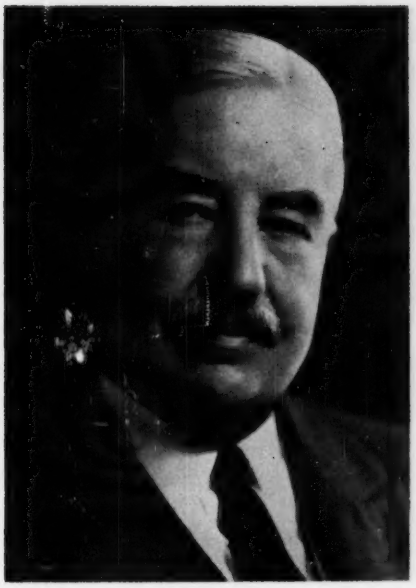
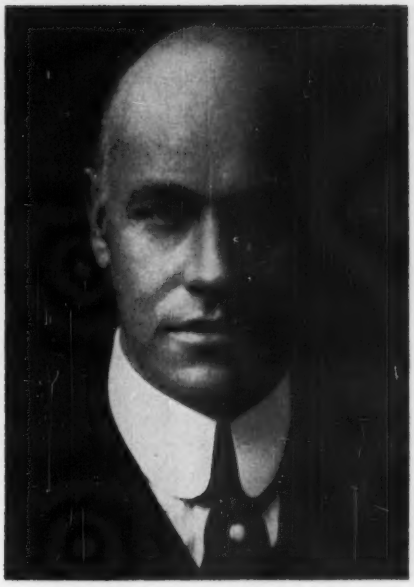


SAMUEL J. MELTZER

The initial suggestion for the founding of the American Society for Clinical Investigation came from Dr. Meltzer in June, 1907, and his was the guiding influence among the group of younger men who laid the actual plans for its organization the following year. His views regarding "what the science of clinical medicine ought to be" formed the basis of the ideals for which the Society has stood during its 50 years of existence. To Dr. Meltzer, therefore, goes the major share of credit for the Society's establishment. Photographs of the eight other "first Young Turks" who, with Dr. Meltzer, comprised the Society's founding committee, appear on the following pages.



JOSEPH A. CAPPS



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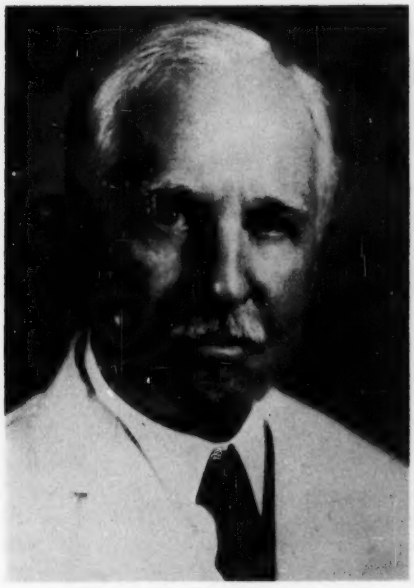
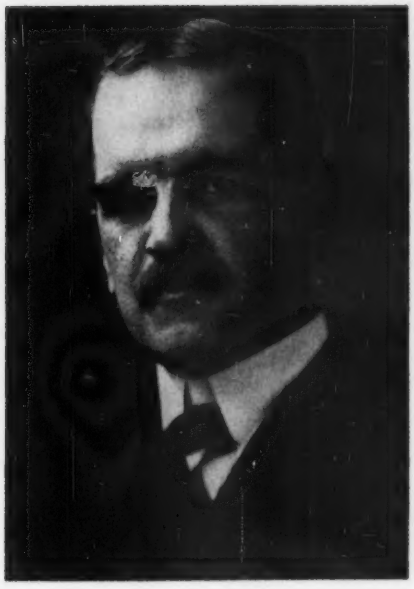


RUFUS COLE

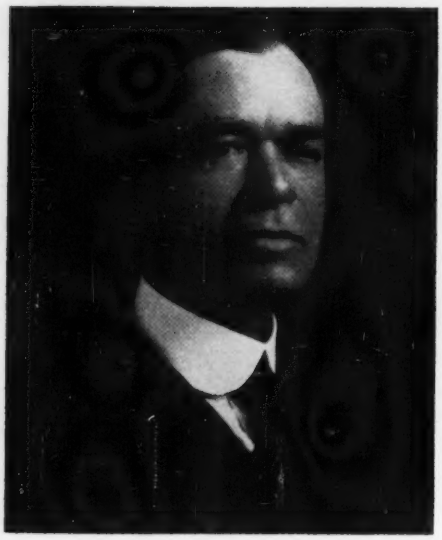


DAVID L. EDSALL

ALBION WALTER HEWLETT

WARFIELD T. LONGCOPE

JOSEPH H. PRATT



WILDER TILESTON

New Willard Hotel, Washington, D. C.,
May 11, 1908.

At 3:30 p.m. the following gentlemen, Drs. D. L. Edsall, S. J. Meltzer, A. W. Hewlett, Wilder Tileston, John Howland, H. A. Christian, W. T. Longcope, R. J. Cole, E. Libman, J. A. Capps, T. C. Janeway, W. J. Calvert, J. L. Miller, J. Sailer, Martin Fischer, E. A. Locke, and Mark Richardson, met in response to a letter of invitation sent out by a committee composed of the following, D. L. Edsall, H. A. Christian, S. J. Meltzer, J. H. Pratt, W. T. Longcope, R. J. Cole, Wilder Tileston, J. A. Capps, A. W. Hewlett, for the organization of a new society whose proposed scope had been outlined in a circular letter.

This meeting was called to order by Dr. D. L. Edsall of Philadelphia.

Dr. Edsall was then elected temporary chairman, Dr. H. A. Christian of Boston temporary secretary.

The Chairman stated that the first thing to be considered was the adoption of a constitution, and that a committee composed of Drs. Longcope, Meltzer, and Christian had prepared a constitution for consideration by the society.

Dr. J. L. Miller of Chicago moved that the constitution be read by single articles. Seconded. Carried.

Dr. T. C. Janeway of New York moved that in order to save time a vote should not be taken

MINUTES OF THE SOCIETY'S CHARTER MEETING

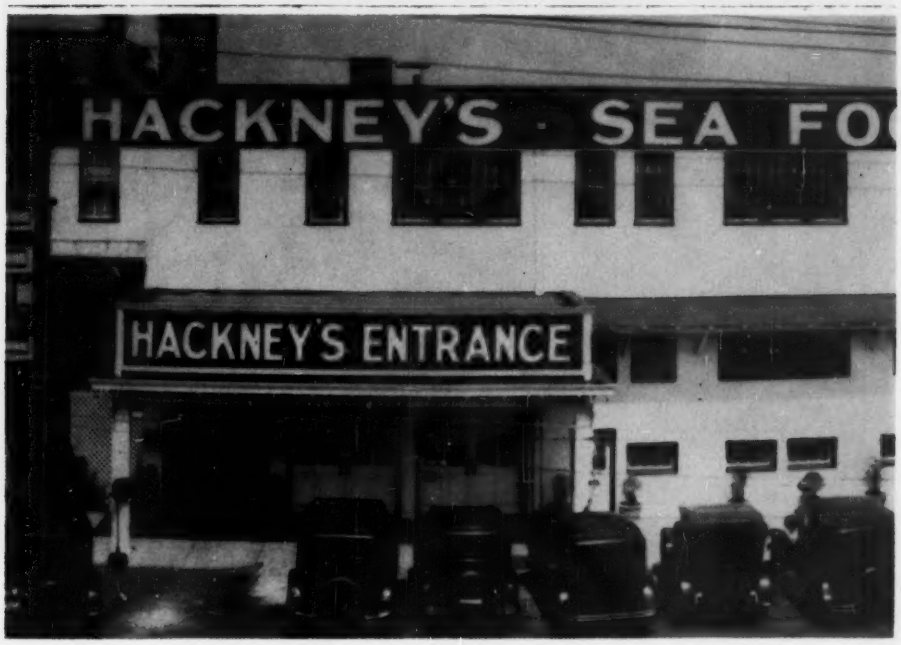
Page 5 from the Society's first record book (there are now five) in which the minutes of the charter meeting of 1908 and all annual meetings until 1922 were recorded in longhand by the secretaries. The page reproduced above was written by Dr. Henry A. Christian who served as secretary from 1908 to 1910.

LANDMARKS

The stretch of Boardwalk in front of the Hotel Traymore (above) was most likely the setting for the informal conversations in which the suggestion for the Society's establishment had its first hearing. It was probably at the Traymore, too, that the group of interested young men gathered in Dr. Meltzer's room to pursue the idea further and to begin formulating plans for the new organization. Between 1917 and 1931, the Society's 12 Atlantic City meetings were held here. The Society was officially constituted at the New Willard Hotel (left) in Washington, the scene of its charter meeting of May 11, 1908. The first and second annual meetings, as well as those of 1915 and 1916 were also convened at the New Willard.

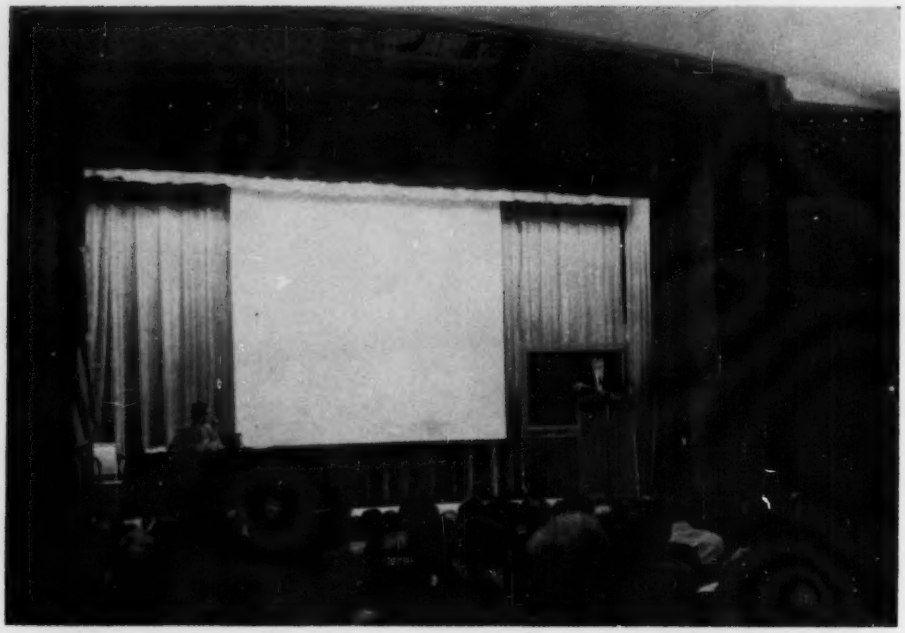
BETWEEN THE ACTS

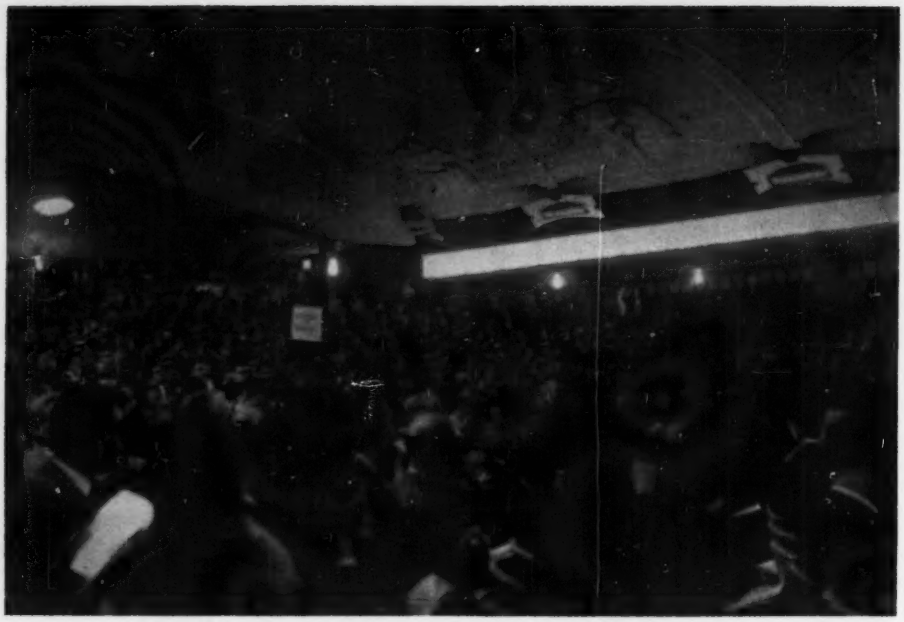
When the mass of new knowledge threatened to cause mental indigestion, the audience often adjourned temporarily to the Boardwalk to discuss, argue, or simply relax before returning for the next paper.



AFTER HOURS: DINNER AND DEBATE

Hackney's Shore Dinners, a traditional eating place for Society members in the "good old days," was the setting for lively post-meeting discussions on Monday evenings after adjournment of the scientific sessions. The photograph above is probably of the 1920's vintage, although Society members have continued to patronize Hackney's more or less en masse up to the present.



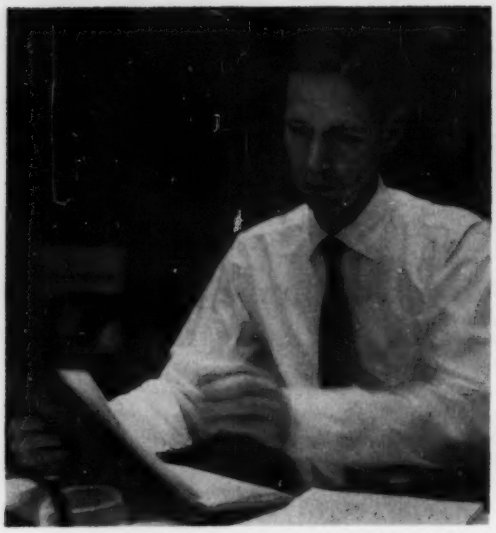


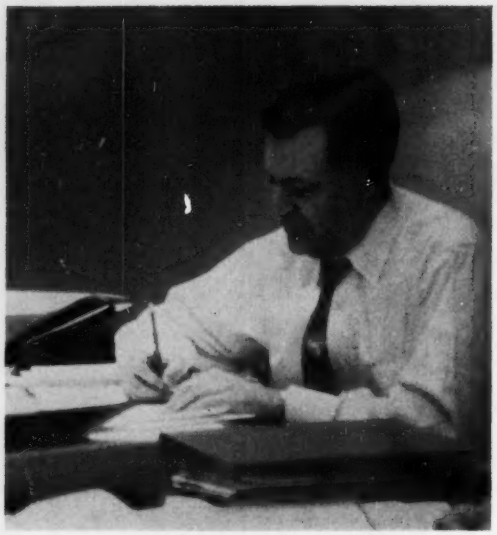
INTIMATE INFORMALITY VS. AUDIENCE ACCOMMODATION

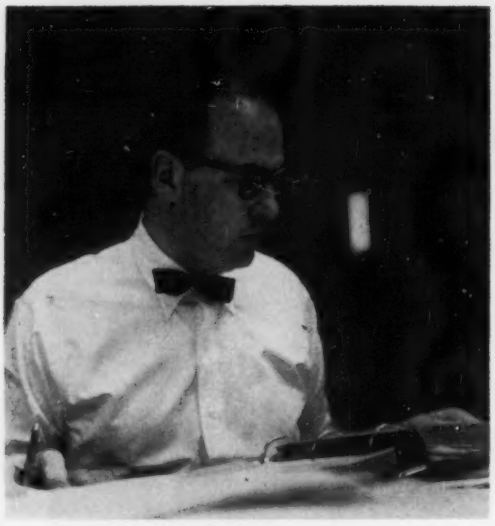
Some idea of the meaning of the controversy over the Society's 1950 move to the Steel Pier Theater can be derived from the views of the two meeting places shown above. At the top is the Viking Room of the Haddon Hall Hotel during one of the 1959 joint Society-American Federation for Clinical Research section meetings. The Viking Room, and the somewhat larger Vernon Room of Haddon Hall were the settings for most of the Society's annual meetings between 1932 and 1950. The prevailing opinion among members active during that period is that these meeting places, in distinct contrast to the auditorium of the Steel Pier Theater (below) provided an atmosphere conducive to informal discussion of papers. The move to the Steel Pier was made, primarily, in response to the large postwar audiences. The picture of the Viking Room was taken from the back row; that of the Steel Pier auditorium, from the front row looking back.

PEOPLE AT AN EXHIBITION

With the move to the Steel Pier, another feature was added to Society programs—the annual scientific exhibits. Displays of scientific instruments and apparatus were introduced on a trial basis for the May meetings of 1952 and 1953 and at the present time are a regularly-scheduled addition to the programs. Exhibits are solicited by a committee of the Society, with final selection being made by the president in a manner similar to the selection of papers for the scientific program. Displays prepared by both commercial and institutional groups have been included. Pictured above is a general view of the exhibit lobby of the Steel Pier Theater (top) and one of the displays included on the 1959 exhibit program (bottom).

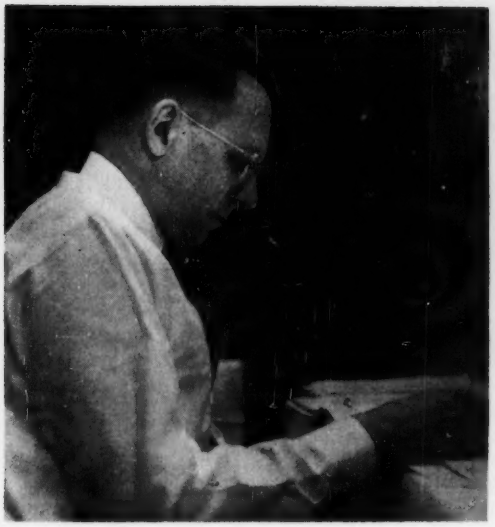


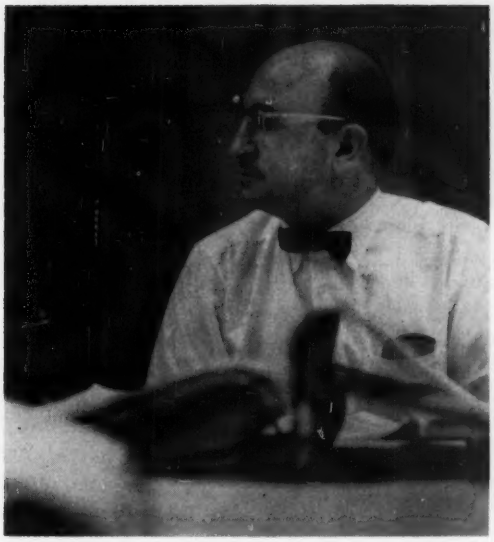




THE COUNCIL OF THE SOCIETY, 1959: OFFICERS

Photographs of the Council were taken during the 1959 meeting. The members are: (top left) Dr. John A. Luetscher, President; (top right) Dr. Saul J. Farber, Secretary-Treasurer; (bottom left) Dr. Vincent P. Dole, Vice President; and (bottom right) Dr. Philip K. Bondy, Editor-in-Chief of the Journal of Clinical Investigation.





THE COUNCIL OF THE SOCIETY, 1959: COUNCILLORS

The councillors, in order of election, are: (top left) Dr. C. Lockard Conley, 1957–1959; (top, right) Dr. John V. Taggart, 1958–1960; and (below) Dr. Harold S. Ginsberg, 1959–1961.

CONSTITUTION OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION (AS AMENDED TO 1959)

ARTICLE I. NAME

The name of the Society shall be "The American Society for Clinical Investigation."

ARTICLE II. OBJECTS

The objects of the Society shall be the cultivation of clinical research by the methods of the natural sciences; the unification of science and the practice of medicine; the encouragement of scientific investigation by the practitioner, and the diffusion of a scientific spirit among its members.

ARTICLE III. MEMBERSHIP

Section 1. *Eligibility.* Any physician residing in the United States or Canada who is less than 45 years old, has accomplished meritorious original investigation in the clinical or allied sciences of medicine and enjoys an unimpeachable moral standing in the medical profession, is eligible to membership.

Section 2. *Nomination and Election.* Nominations to membership may be made in writing to the Secretary at any time. Such nominations must be signed by two members, active or emeritus, but not by officers of the Society during their tenure of office. Each nomination must be accompanied by (1) a statement from *each* of the sponsors as to the qualifications of the nominee and (2) a list of the academic degrees, professional positions and publications of the nominee. All names thus placed in nomination prior to January 1st preceding each annual meeting shall be considered by the Council, which shall pass upon the qualifications of the candidates and report to the Society for election at the annual meeting such names as it shall approve by a two-thirds vote. An affirmative vote of at least three-fourths of the members present shall be necessary for election.

Section 3. The Council shall have authority to recommend each year to the Society the names of properly qualified candidates equal to the number of retiring Active Members, or up to the number of thirty-five, depending on which number is the larger.

The Council shall also report to the Society a list of candidates whose names shall be continued in nomination.

Section 4. *Obligations.* Members must manifest a continuous interest in the objects of the Society by the following: (1) They must attend at least every other annual meeting. (2) They must be active in the diffusion of the principles of the Society, particularly among their students and professional associates.

Failure to attend two consecutive meetings without an explanation acceptable to the Council carries with it forfeiture of membership.

Members shall be subject to annual dues as set forth in Article IX.

Section 5. *Privileges.* Members may participate in the business and scientific sessions of the Society and each may submit or sponsor one paper for the scientific program of each annual meeting. Only members are eligible to hold office in the Society.

Section 6. *Expulsion.* Members may be expelled also for reasons not especially mentioned in the Constitution by a three-fourths vote of the total membership. The vote shall be by secret ballot.

Section 7. *Emeritus Members.* Members who have reached their forty-fifth birthdays prior to the annual spring meeting of the Society shall automatically become Emeritus Members at the end of that meeting. Members whose membership has extended throughout five years or more may at their own request be transferred by the Council to Emeritus Membership.

Section 8. Each Emeritus Member shall be entitled to attend all meetings, participate in the business and scientific discussion but may not vote or hold office. With respect to the scientific program, an Emeritus Member may introduce one paper by non-members, but may not appear as

author or co-author. He may also nominate members. He shall be exempt from dues.

ARTICLE IV. Officers

Officers of the Society shall be a President, Vice-President, and Secretary-Treasurer, all to be elected annually. The Editor-in-Chief of the Journal of Clinical Investigation shall be an ex-officio member of the Council of the Society. His maximum tenure of office shall be 5 years, and his successor shall be elected, when possible, one year prior to his retirement. In addition each year a Councillor shall be elected to serve three years. However, at the first meeting three Councillors shall be elected, one of whom shall serve one year, one two years, and one three years. The order of retirement of the first Councillors is to be determined by lot. All officers are ineligible for re-election except the Secretary-Treasurer, who may be re-elected twice, the total period of continuous service not to exceed three years. These officers are to be nominated by a committe of three appointed by the President. The election shall be by secret ballot.

ARTICLE V. Duty of Officers

The duties of President, Vice-President, and Secretary-Treasurer shall be those usually assigned to these officers. The Secretary-Treasurer shall also arrange for the publication of abstracts of papers presented at the meetings of the Society.

ARTICLE VI. Council

The Council shall consist of the President, Vice-President, Secretary-Treasurer, Editor of the Journal of Clinical Investigation and the three Councillors. Four members shall constitute a quorum.

It shall be the duty of the Council to supervise the affairs of the Society, to make all the arrangements for the annual meeting, to report on the work of the members, to consider all nominations for membership and to report on them at the meeting at which they shall be balloted on.

There shall be an Editorial Committee of the Society consisting of 15 members not more than one-third of whom shall be emeritus when appointed; these members shall be elected by the Society upon recommendation of the Editor-in-Chief in collaboration with the Editorial Committee and with the approval of the Council. The members shall be elected for 5 years with three such appointments being made annually. Initially, the appointments will necessarily require the proper staggering.

A chairman of this Editorial Committee shall be elected annually from among its members, and by its members.

The Editorial Committee shall be responsible for the editorial policies of the Journal and the nomination of the Editor-in-Chief. They shall individually and collectively be available to assist the Editor-in-Chief at his request, in any matters.

In addition, Associate Editors shall be appointed by the Editor-in-Chief and these Associate Editors shall sit with the Editorial Committee at all regular meetings.

ARTICLE VII. Meetings

The Society shall hold one general meeting annually.

ARTICLE VIII. Quorum

Any number of members at the appointed time of the annual meeting shall constitute a quorum for the transaction of ordinary business. For the election of members fifteen shall be necessary for a quorum, and for altering the Constitution forty per cent of the membership shall be necessary.

ARTICLE IX. Annual Dues

The annual dues of the Society shall be such sum as the Council from time to time may fix. The non-payment of dues for two consecutive years carries with it the forfeiture of membership.

ARTICLE X. Amendments to the Constitution

Amendments to the Constitution must be proposed in writing by five members at an annual meeting, and must be acted upon at the succeeding annual meeting, notices of which shall contain an announcement of the proposed amendment, and such amendments shall require for their adoption an affirmative vote of three-fourths of those present.

OFFICERS OF THE SOCIETY FROM ITS ORGANIZATION TO THE PRESENT TIME

Year	*President*	*Vice-President*	*Treasurer*	*Secretary*	*Councillors*		
1909	Meltzer, S. J.	Edsall, D. L.	Howland, J.	Christian, H. A.	Miller, J. L.	Janeway, T. C.	Hewlett, A. W.
1910	Edsall, D. L.	Capps, J. A.	Howland, J.	Christian, H. A.	Janeway, T. C.	Hewlett, A. W.	Fischer, M.
1911	Pratt, J. H.	Longcope, W. T.	Miller, J. L.	Tileston, W.	Hewlett, A. W.	Fischer, M.	Thayer, W. S.
1912	Janeway, T. C.	Sailer, J.	Blumer, G.	Tileston, W.	Fischer, M.	Thayer, W. S.	Cole, R. I.
1913	Thayer, W. S.	Hewlett, A. W.	Bogga, T. R.	Tileston, W.	Thayer, W. S.	Cole, R. I.	Capps, J. A.
1914	Miller, J. L.	Longcope, W. T.	Foster, N. B.	Morris, R. S.	Cole, R. I.	Capps, J. A.	Howland, J.
1915	Cole, R. I.	Howard, C. P.	Foster, N. B.	Morris, R. S.	Capps, J. A.	Howland, J.	Hewlett, A. W.
1916	Capps, J. A.	Tileston, W.	Hamman, L. V.	Peabody, F. W.	Howland, J.	Hewlett, A. W.	Longcope, W. T.
1917	Emerson, H.	Foster, N. B.	Robinson, G. C.	Peabody, F. W.	Hewlett, A. W.	Longcope, W. T.	Slemons, J. M.
1918	Blumer, G.	Joslin, E. P.	Hess, A. F.	Palmer, W. W.	Longcope, W. T.	Slemons, J. M.	Irons, E. E.
1919	Christian, H. A.	Robinson, G. C.	Hess, A. F.	Palmer, W. W.	Slemons, J. M.	Irons, E. E.	Blumer, G.
1920	Longcope, W. T.	Woodyatt, R. T.	Hess, A. F.	Palmer, W. W.	Irons, E. E.	Blumer, G.	Lee, R. I.
1921	Rowntree, L. G.	Cowie, D. M.	Pemberton, R.	Means, J. H.	Blumer, G.	Lee, R. I.	Swift, H. F.
1922	Woodyatt, R. T.	McLester, J. S.	Pemberton, R.	Means, J. H.	Lee, R. I.	Swift, H. F.	Newburgh, L. H.
1923	Joslin, E. P.	Hoover, C. F.	Pemberton, R.	Means, J. H.	Swift, H. F.	Newburgh, L. H.	Marriott, W. M.
1924	Howard, C. P.	Cohn, A. E.	Barr, D. P.	Austin, J. H.	Newburgh, L. H.	Marriott, W. M.	Means, J. H.
1925	Hewlett, A. W.	McCann, W. S.	Barr, D. P.	Austin, J. H.	Marriott, W. M.	Means, J. H.	Irons, E. E.
1926	Bass, C. C.	DuBois, E. F.	Barr, D. P.	Austin, J. H.	Means, J. H.	Irons, E. E.	McCann, W. S.
1927	DuBois, E. F.	Smith, F. M.	Harrop, G., Jr.	Wearn, J. T.	Irons, E. E.	McCann, W. S.	Musser, J., Jr.
1928	Swift, H. F.	Strouse, S.	Harrop, G., Jr.	Wearn, J. T.	McCann, W. S.	Musser, J., Jr.	Graham, D.
1929	Meakins, J. C.	Gamble, J. L.	Harrop, G., Jr.	Wearn, J. T.	Musser, J., Jr.	Graham, D.	Addis, T.
1930	Addis, T.	Peters, J. P.	Harrop, G., Jr.	Sturgis, C. C.	Graham, D.	Wilder, R. M.	Wearn, J. T.
1931	Blake, F. G.	Robertson, O. H.	Holt, L. E., Jr.	Sturgis, C. C.	Wilder, R. M.	Wearn, J. T.	Bloomfield, A. L.
1932	Rivers, T. M.	Wearn, J. T.	Holt, L. E., Jr.	Sturgis, C. C.	Bock, A. V.	Bloomfield, A. L.	Peters, J. P.
1933	Bloomfield, A. L.	Burwell, C. S.	Holt, L. E., Jr.	Blumgart, H. L.	Loeb, R.	Bock, A. V.	Hayman, J. M., Jr.
1934	Burwell, C. S.	Loeb, R.	Paul, J. R.	Blumgart, H. L.	Bock, A. V.	Hayman, J. M., Jr.	Tillett, W. S.
1935	Wearn, J. T.	Sturgis, C. C.	Paul, J. R.	Blumgart, H. L.	Hayman, J. M., Jr.	Tillett, W. S.	Bauer, W.
1936	Loeb, R.	West, R.	Paul, J. R.	Hayman, J. M., Jr.	Tillett, W. S.	Bauer, W.	Mettier, S.
1937	Tillett, W. S.	Holt, L. E., Jr.	Paul, J. R.	Hayman, J. M., Jr.	Bauer, W.	Mettier, S.	McIntosh, R.
1938	Paul, J. R.	Jackson, H., Jr.	Andrus, E. C.	Hayman, J. M., Jr.	Mettier, S.	McIntosh, R.	Harrison, T. R.
1939	Harrison, T. R.	Miller, C. P.	Andrus, E. C.	Starr, I.	McIntosh, R.	Turner, R.	Castle, W. B.
1940	Starr, I.	Blumgart, H. L.	Andrus, E. C.	Landis, E. M.	Turner, R.	Castle, W. B.	McEachern, D.
1941	Castle, W. B.	Warren, S. L.	Thompson, W. P.	Landis, E. M.	Dock, W.	McEachern, D.	Keefer, C. S.
1942	Dock, W.	Hayman, J. M., Jr.	Thompson, W. P.	Landis, E. M.	McEachern, D.	Keefer, C. S.	Smith, D. T.
1943	Landis, E. M.	Horsfall, F. L.	Janeway, C. A.	Spink, W. W.	Keefer, C. S.	Smith, D. T.	Wilbur, D. L.
1944	Albright, F.	McKhann, C. F.	Janeway, C. A.	Spink, W. W.	Smith, D. T.	Wilbur, D. L.	Finland, M.
1945	Long, C. N. H.	Watson, C. J.	Janeway, C. A.	Spink, W. W.	Wilbur, D. L.	Finland, M.	Stead, E. A.
1946	Francis, T.	Browne, J. S. L.	Wilkins, R. W.	Stead, E. A., Jr.	Finland, M.	Ferris, E. B.	Lockwood, J. S.
1947	Watson, C. J.	Salter, W. T.	Wilkins, R. W.	Stead, E. A., Jr.	Ferris, E. B.	Lockwood, J. S.	Wood, W. B.
1948	Browne, J. S. L.	Finland, M.	Wilkins, R. W.	Stead, E. A., Jr.	Lockwood, J. S.	Wood, W. B.	Burch, G. E.
			Secretary-Treasurer				
1949	Spink, W. W.	Ham, T. H.	Beeson, P. B.		Wood, W. B.	Burch, G. E.	Bean, W. B.
1950	Ham, T. H.	Ferris, E. B.	Beeson, P. B.		Burch, G. E.	Bean, W. B.	Bradley, S. E.
1951	Ferris, E. B.	Thorn, G. W.	Beeson, P. B.		Bean, W. B.	Bradley, S. E.	Conn, J. W.
1952	Wood, W. B.	Janeway, C. A.	Kirby, W. M. M.		Bradley, S. E.	Conn, J. W.	Harvey, A. M.
1953	Stead, E. A., Jr.	Moore, C. V.	Kirby, W. M. M.		Conn, J. W.	Harvey, A. M.	Rammelkamp, C. H.
1954	Moore, C. V.	Beeson, P. B.	Kirby, W. M. M.		Harvey, A. M.	Rammelkamp, C. H.	Darby, W. J.
1955	Williams, R. H.	Burch, G. E.	Myers, J. D.		Rammelkamp, C. H.	Darby, W. J.	Moore, F. D.
1956	Harvey, A. M.	Rammelkamp, C. H.	Myers, J. D.		Darby, W. J.	Moore, F. D.	Rantz, L. A.
1957	Bradley, S. E.	Ebert, R. V.	Myers, J. D.		Moore, F. D.	Rantz, L. A.	Conley, C. L
1958	Ebert, R. V.	Luetscher, J. A., Jr.	Farber, S. J.		Rantz, L. A.	Conley, C. L.	Taggart, J. V.
1959	Luetscher, J. A., Jr.	Dole, V. P.	Farber, S. J.		Conley, C. L.	Taggart, J. V.	Ginsberg, H. S.
1960	Berliner, R. W.	Mudge, G. H.	Farber, S. J.		Taggart, J. V.	Ginsberg, H. S.	Cartwright, G. E.

Roster of the

American Society for Clinical Investigation

1959

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HISTORY OF THE JOURNAL OF CLINICAL INVESTIGATION, 1924–1959

I. PERSONNEL AND POLICIES

By ELLEN R. BRAINARD

The Journal of Clinical Investigation bears on its letterhead the statement "Published and Edited by the American Society for Clinical Investigation." When its establishment was first suggested, however, there was at least an even chance that it would be a journal "published and edited by the Rockefeller Institute" instead. By the time the first reference to the Journal appeared in Society records—in 1924—its existence was an all but established fact, awaiting only the publication of a first issue to make it a reality. Plans, obviously, had been afoot considerably earlier. A small group of Society members—G. Canby Robinson, Rufus Cole, J. Harold Austin, Alfred Cohn, to mention only the principals—had begun to discuss the idea informally perhaps as early as 1916 and certainly by 1920. Dr. Robinson (1) relates the story of the Journal's origin as follows:

> As I recall, the idea of starting a journal originated . . . among the members who were then disturbed by the length of time, usually a year or more, between the submission of a paper and its publication. The principal journals available for the publication of papers on scientific clinical studies were the Archives of Internal Medicine and the American Journal of the Medical Sciences, although some of us published papers in the English journal, Heart, and in the Journal of Experimental Medicine.

All of these men were in accord regarding the desirability of founding a new journal for clinical research. The problem was finding a sponsor. In 1921, Dr. Cole submitted a proposal for the establishment of such a journal to the Rockefeller Institute's Board of Scientific Directors. His plan, set forth in a 14 page memorandum (2), called for a journal representing the work of the medical clinics to be published by the Institute as a kind of companion volume to the Journal of Experimental Medicine which represented, primarily, the work of the laboratories of the basic sciences.[1]

In the introductory paragraph of the memorandum, Dr. Cole notes that he had presented a similar proposal to the Board some five years earlier—about 1916—although at that time its members had considered the establishment of a journal for clinical research inadvisable. The 1921 report, Dr. Cole said, was prepared on the assumption that subsequent developments in clinical medicine had been such as to warrant reconsideration of a journal devoted to this field. Regarding these developments, his report read as follows:

> Up to within a very recent period the medical clinics of this country have taken a relatively unimportant part in the development of the science of medicine. While isolated observations of great value have been made by men engaged in clinical teaching and practice, relatively few consciously organized scientific investigations of medical problems have been undertaken in our university departments of medicine or by men trained in the study of disease at the bedside. Scientific medicine in America has developed in the laboratories of pathology, of physiology, of physiological chemistry, and of pharmacology. This is well shown by a review of the scientific medical journals of this country and is in marked contrast to the conditions in Germany, for instance, where for the past 80 years or more the clinics of medicine have taken an important part in the transformation of medicine from an empiric practice into a scientific discipline.
>
> During the past 10 years, however, there has been a well marked movement in this country directed toward the elevation of the departments of medicine of the universities into a position more nearly approaching that which it is believed they should occupy on account of their natural relation to the phenomena with which they deal. This has been shown by the appointment of full time staffs and by the establishment in the departments of medicine of laboratories in which investigations can be carried on by the methods which have been developed in the more fundamental sciences. It is therefore felt that the time is imminent when these modern clinics of medicine will seek expression in journals which take cognizance of this modern development.

In support of his contention that there would be ample material to fill the pages of a journal such as he proposed, Dr. Cole specified a number of university departments from which work of a high quality was already forthcoming and noted,

[1] A copy of the memorandum was kindly provided by Dr. Cole.

furthermore, that "from the Hospital of the Rockefeller Institute alone, papers sufficient to fill nine volumes" had appeared during the previous 10 years. He also directed attention to the Society as a potential source of material. In this connection, the following excerpt is of particular interest:

> The American Society for Clinical Investigation has brought together a large number of the younger clinicians engaged in scientific investigation and a considerable proportion of the papers now presented at the annual meeting of this society is of sufficiently high grade to justify publication in this journal. . . . It is believed by a considerable number of the members of this Society that it should have an independent journal for the publication of its contributions. Experience, however, has shown that the best results are not obtained by placing scientific journals in the control of societies, with their changing staffs of officers and shifting ideals. The establishment of a clinical journal by the Rockefeller Institute, it is believed, would meet the needs of the Society for Clinical Investigation.

Dr. Cole discussed his proposal with the Institute's Board of Scientific Directors in April, and again in November, 1921.[2] On both occasions, although response was favorable, no action was taken and more than a year elapsed before any further mention of the journal appeared in Institute records. Meanwhile, however, activity on other fronts had gone on apace. Of the turn of events which took place during this interim period, Dr. Cole (3) writes as follows:

> I had already (by April, 1921) been in communication with Canby Robinson and others and discussed with them the possibility of publishing the journal by the Society, with a subsidy from the Institute. At the time, Dr. Flexner was finding the publication and editing of the Journal of Experimental Medicine a great burden, and he thought the Institute should not take on any further responsibilities of this kind. That the Journal should be published by the Society with a grant from the Institute was entirely agreeable to me and to the others who were interested. But there were delays in having the Board of Scientific Directors commit themselves and during this waiting period it was Canby Robinson who pushed the matter along and made it possible to start the journal.

Dr. Robinson and his colleagues were given what amounted to *ex post facto* authority to negotiate with representatives of the Rockefeller Institute when, on Sept. 15, 1923, Society President C. P. Howard appointed a committee headed by Dr. Robinson and consisting of Drs. Austin, Cohn, Cole, Warfield Longcope, Francis Peabody and Rollin T. Woodyatt to study the formation of a new journal which could be affiliated with the Society should it approve at the next annual meeting. Institute authorities agreed to subsidize the journal, final plans were drawn up, and at its meeting of May 5, 1924, the Society heard and approved the Robinson committee's report which provided for the establishment of "a journal to be called The Journal of Clinical Investigation" and to be made "the property and organ" of the Society.

As outlined in this report and detailed in subsequent records, the editorial organization of the Journal was to consist of an Editorial Committee, an Editorial Board, and the editor-in-chief. The 15 man Editorial Committee was designed as the Journal's policy making body, its members to act as "advisory editors" and to have authority "in matters pertaining to . . . general policy." The Editorial Board, which consisted initially of six members, was to be the Journal's managerial body. In his 1921 proposal, Dr. Cole had urged that editorial responsibility be exercised not by a single individual but by a group of four or five editors, each representing a special field of clinical medicine. This recommendation, essentially, was incorporated in the Journal's plan of operation. The editor-in-chief was to function primarily as a co-ordinator. Upon receipt of a manuscript, he would assign it to that Board member best qualified by his scientific interests to evaluate it. The Board member, in turn, was to determine the paper's suitability for publication and, in the case of acceptance, to assume complete responsibility for editing it.

The foregoing plan for the Journal's organization, as submitted to and approved by the Society, was complete down to the naming of the initial Committee and Board members and of Dr. Robinson as editor-in-chief. Thus, immediately upon receiving the green light from the Society, forces were mobilized and the plan put into effect. The first number of the new journal was dated Oct. 20, 1924. Subsequent numbers were issued in December, 1924, and in February, April, June and August, 1925, thereby bringing the first vol-

[2] Information on the progress of Dr. Cole's proposal through Rockefeller Institute channels was kindly provided by Mr. Eugene Kone, Public Relations Consultant, the Rockefeller Institute, New York, N. Y.

ume to a close. Its 605 pages contained 30 scientific articles, the proceedings of the Society's 1925 meeting, an index, and an article entitled "Purposes in Medical Research: An Introduction to the Journal of Clinical Investigation," by Dr. Cohn.[2]

Dr. Robinson continued as editor-in-chief through the publication of the first two volumes, terminating his editorship with the close of the second in August, 1926. During the following year, he worked closely with his successor, Dr. Austin, who held office until mid-1935.

The dominant fact of Journal life during these years—and up to the early 1940's—was financial insecurity. Only once (in 1934) was it so much as suggested that the brave new publication be abandoned altogether for lack of funds, but the Journal's first 15 years were shaped by a continual struggle to make ends meet. In one respect, at least, the hopes of its founders were fully justified: there was material aplenty to fill its pages. Although in May, 1927, Dr. Robinson reported that "a large majority" of the papers submitted had been published and urged Editorial Committee members to direct appropriate material from their departments to the Journal, by the following year manuscripts were flowing in rapidly enough to warrant increasing the number of pages from 600 to approximately 900 per year. By 1929 a considerable backlog of material had accumulated. The ever-present threat of destitution, however, thwarted hopes for further expansion. As Editor Austin told the Board in May, 1929:

> In spite of this press of material, I advise against any increase in the rate of publication until all annual deficit at the present rate . . . has been eliminated.

The "annual deficit" was attributable, primarily, to the brevity of the subscription list which, by May, 1925, amounted to a pathetic 278 as against the 500 predicted and the 1,000 estimated as necessary for self-support. Two years later, although the total had risen above 300, Dr. Austin dolefully told the Council, "We cannot hope for more than 700 to 800 subscribers eventually." But "eventually" was to be a long way off. After 10 years of publication the subscription list barely topped 550; after 15, it was an improved, but still meager, 900. Commenting on the Journal's rather disappointing reception, Dr. Austin in his 1928 report said:

> The small size of the subscription list may probably be explained as follows: The published results of scientific clinical investigation such as appear in the Journal are of the utmost importance and value to those engaged in our university clinics in similar investigations. . . . The recognition of the value of these articles by physicians at large in our country must inevitably, however, take much more time and can probably be expected only after the practical results of such studies become demonstrated. Even then, it may prove that the original studies on which results rest may have interest for only a limited group. Furthermore, in our university clinics where library facilities are now well developed, many workers are supplied by the library copy of the Journal, so that the number consistently using (it) is undoubtedly larger than the number of subscribers. All of the important medical and university libraries in this country and Canada are subscribers to the Journal.

Thus, until 1940, the Journal subsisted only by virtue of generous contributions from external sources. Appropriations from the Rockefeller Institute, totaling $15,000, carried it through the publication of its fifth volume, completed in June, 1928. At that time, although the Institute continued to make sizable annual contributions until as late as 1936 (amounting to an additional $2,200), it withdrew as the Journal's major provider. In anticipation of this withdrawal, the editors had begun casting about for new benefactors. Negotiating with Abraham Flexner, Drs. Cohn and Austin were able to secure a $4,500 grant for 1928–29 (of which about one-third was refunded as unexpended balance at the end of the year) from the Rockefeller Foundation's General Education Board. Further funds were made available when, at the request of the editors, the Council in 1928 raised the dues of active members from $3 to $10 per year with the $7 difference going into Journal coffers and active members, in return, being automatically added to the ranks of Journal subscribers. (In 1935, another crisis year, emeritus members' dues were set at $7 and they, too, became "compulsory" Journal readers.)

A second measure enacted at the 1928 meeting was aimed at enlisting the support of the university departments and clinics with which Society members were affiliated. To encourage their assistance, a constitutional amendment creating the status of "associate membership" in the So-

[2] This article is discussed briefly in the section dealing with the history of the Society, Chapter III.

ciety was introduced. It was to be open to universities and clinics upon payment of annual dues (fixed by the Council at $200) which would be used entirely for Journal support. Although only four (Chicago, Michigan, Yale and Western Reserve) accepted such standing in the Society, 19 different clinics plus several sympathetic societies (the American College of Physicians, the Central Interurban Clinical Club, and the Central Society for Clinical Research) poured a total of almost $15,500 into the Journal treasury between 1928 and 1936.

In 1931, largely through the efforts of Dr. Robinson, an arrangement was worked out with the Chemical Foundation whereby that organization agreed to assume responsibility for the conduct of Journal business affairs, an arrangement which remained in effect for nearly 10 years. During this period, the Foundation contributed a total of $3,400 in outright subsidy and, in addition, bore the operating costs of editorial and business offices, including secretarial salaries. Had it not been for the depression, perhaps the high hopes for rapid improvement of the Journal's financial position entertained in 1931 by both Journal and Foundation authorities could have been realized. As it was, the situation, by 1934, was extremely grave. A letter from Dr. Austin to Journal and Society officers, dated April 5, 1934, depicted the Journal's position as follows:

> In consequence of the shrinking of support from . . . our clinics the budget of the Journal is becoming less nearly balanced each year. To date this shrinkage has been met by payments from our bank reserve so that the deficit met annually by the Chemical Foundation has not increased. The remaining bank reserve, $3,084.12, however, can only last, at the present rate of issue, for another year or 18 months.
>
> Furthermore, the Chemical Foundation has notified us that economic requirements compel the Foundation to recognize limits in its support of publications. While it has set no definite limit with respect either to the amount of funds or the duration of its support . . . it has indicated to us that a journal to receive continuing support must be progressing toward a self-supporting basis. It is impossible to contend that under our present policies our Journal is exhibiting satisfactory progress toward self-support.

After outlining several possible courses of action, Dr. Austin concluded on what is perhaps the most pessimistic note in Journal history:

> The course which is obviously the easiest and which we will follow perforce if no other experimentation is attempted is to continue as at present until our bank reserves are exhausted bringing the Journal to a close, I venture to predict, at about December, 1935.

Fortunately, "other experimentation" was attempted. With the enactment of several economy measures and the Foundation's continued, if reluctant, support, the Journal survived and, as prosperity returned to the nation, Journal fortunes began to improve.

During 1935, the editorship changed hands twice. In May of that year, Dr. Austin, having served the Journal continuously from the time its formation was first suggested, tendered his resignation as editor-in-chief. Dr. John R. Paul was unanimously elected to the position and took office about July 1, 1935, but due to illness, he was forced to resign on July 21, 1935. Dr. Randolph West then accepted the editorship and held office until mid-1941.

In July, 1940, Chemical Foundation officers notified Dr. West that although they were willing to continue managing the Journal's business affairs, the Foundation would not be able to meet any future deficit, no matter how small. Correspondence among Editorial Board and Committee members and officers of the Society resulted in a decision to terminate the Journal-Foundation relationship and to merge the offices of Society treasurer and Journal business manager. The task of reorganizing the Journal's business affairs thus fell to Dr. West and to Society Treasurer, William P. Thompson. On Oct. 1, 1940, an agreement was signed with the president of the Chemical Foundation effecting a final closing of accounts.

It was at this time that the Journal "adopted a policy of accepting reputable advertising." The implication that this was a new policy seems a little puzzling in light of the fact that the original plan of operation had charged the editor-in-chief with responsibility for soliciting and approving advertisements, and that later the Chemical Foundation had agreed to secure advertisers. It is doubtful that the Journal ever refused advertisements as a matter of policy. Most likely, the practice of accepting them had simply died through inability to interest manufacturers of medical apparatus and supplies in a publication

with so few subscribers. In any case, in late 1940, Dr. West undertook to re-introduce advertising into the Journal's pages. The net profit of approximately $1,100 made from advertising in 1941 was a significant component of the overall surplus with which the Journal ended that year.

In May, 1941, Dr. James L. Gamble was elected to succeed Dr. West and, during the summer, Journal headquarters was transferred from New York to Boston. The following year, Dr. Thompson retired as treasurer-business manager. On his recommendation, a successor was chosen with a view to his year-round proximity to the editor in order to facilitate the frequent consultation which experience had shown to be essential. Thus, in May, 1942, Dr. Charles Janeway of Boston was elected treasurer and the Journal's business management was re-united with its editorial offices. Testimony to the growing size of Journal operations was the creation, at Dr. Gamble's request, of the office of associate editor to which Dr. Allan M. Butler was appointed in May, 1941. Three years later, the Council authorized the appointment of two additional associates to help with the "on-the-spot" management of editorial affairs. Drs. Chester S. Keefer and Eugene M. Landis, both of Boston, were named at that time. The conduct of business operations, too, had become an exceedingly time-consuming task. Accordingly, when his term expired in 1945, Dr. Janeway recommended that the Society treasurership be separated from the position of Journal business manager. Dr. Robert Wilkins was elected to the former office and Dr. Janeway continued in the latter until 1947.

The war presented a number of special problems, as well as some unique opportunities, in medical journalism. During the early years of the conflict, it was anticipated that there might be some reduction in the normal inflow of manuscripts suitable for publication since many of the Journal's regular contributors were engaged in special studies of military relevance. Although the total number of incoming manuscripts declined in 1943 and 1944, Dr. Gamble's reports indicate that the number of acceptable papers was fairly well sustained. At the same time, additional material was made available through the work of military study groups. Much of this, of course, was classified, but in some cases speedy publication was considered so desirable that the Journal was able to obtain grants for the express purpose of making the results available at an early date. During 1943 and 1944, publication of a number of reports of work done under contract with the Office of Scientific Research and Development was financed by funds from the Josiah Macy, Jr. Foundation. One of these was a 23 paper series entitled "Chemical, Clinical and Immunological Studies on the Products of Human Plasma Fractionation" published in July, 1944. This, in effect, was the Journal's first "supplement." Such additions to the regular issue, while entailing no extra cost, did give rise to another problem—the wartime paper shortage. Dr. Gamble's 1944 report contained the following rather amusing account of Journal efforts to circumvent it:

> Your innocent editor was . . . given pause by discovering the corollary that these additional pages were in excess of our paper quota as prescribed by the WPB. With an even larger inflow of material in prospect this year, it became necessary to develop the temerity to assail WPB for more paper. Two offensives were necessary. The first one was beaten back but the second one succeeded. The Journal did not emerge from this conflict without scars. For instance, it was obliged to submit to the indignity of being called a magazine. Our commander in the field to whose durable valor we owe the paper which will carry us through the year was Dr. Coke Andrus. The Journal offers him thanks and admiration without limit.

This was only a temporary difficulty, however. Beginning in 1945, additional wartime studies found their way into the Journal through a $6,000 grant from the Commonwealth Fund which helped to defray the cost of such supplements as an 11 paper series on "Clinical Uses of 2,3-Dimercaptopropanol (BAL)" in July, 1946, and a 23 paper "Symposium on Malaria" in May, 1948.

A significant policy innovation made by Drs. Gamble and Butler was that of asking Society members at large, irrespective of their membership on the Editorial Board, to review manuscripts. The reasons for the change, and the advantages which seemed to accrue to it, are explained in the following excerpt from Dr. Gamble's 1942 report:

> (The new policy) consists of sending papers for review to members of the Society, active or emeritus, se-

lected on the basis of their experience with the subject with which a paper deals. Obviously this plan greatly reduces the number of papers reviewed by members of the Editorial Board. The lines of approach to disease and especially the technics employed have become so many and various as to make this plan seem necessary. We are not conscious of any disrespect to the Board in pursuing it. The Board would have to be several times larger than it is to provide an expert referee for each paper. We also think that we have discovered another and perhaps larger reason. We feel that it would be desirable for the active members to realize more than they do that the Journal is an undertaking of the Society and a very important one.

This "wide open" reviewing policy was endowed by Journal and Society officers in 1942 and thereafter became standard Journal practice. Under the next editor, Dr. Eugene B. Ferris, the system was considerably extended. By requiring authors to submit papers in duplicate, the editors were able to obtain two evaluations of each manuscript. An increasing number of these were solicited from non-members, thus enabling the Journal to seek the advice of a group of critics more numerous and more diversified in scientific specialties than that afforded by the membership alone. A growing proportion of manuscripts were returned to the authors for revision and it soon became apparent that the new reviewing policy had added another dimension to the Journal's editorial functions, that of providing contributors with critical evaluation of their work over and above the judgment implied in the rejection or acceptance of a paper.

Dr. Gamble presented his resignation to the Council at the 1946 meeting. The appointment of Dr. Eugene B. Ferris of Cincinnati was approved by the Society in May, 1947, and in midsummer of that year, the Journal changed hands. Dr. Gamble's term, which had begun shortly after the reorganization of the Journal's business management, ended with another major reorganization, this one involving the editorial department. Between the May meetings of 1946 and 1947, a committee under the chairmanship of Dr. George Thorn which had been appointed "to review and clarify all aspects of the relationship between the Society and the Journal" worked with Dr. Gamble and Editor-Elect Ferris in conducting a thorough reappraisal of Journal structure and policies. In substance, the committee's recommendations, presented in the form of constitutional amendments and adopted by the Society in 1948, were as follows:

1. The Editorial Board should be abolished.
2. Policy-making and reviewing responsibilities should be combined in a redesigned Editorial Committee consisting of 15 members of whom no more than one-third should be emeritus at the time of appointment.
3. The editor-in-chief should be an ex officio member of the Council and, in order to keep the number of Council members the same, the offices of Society secretary and treasurer should be combined. The editor should serve a maximum of five years and a successor should be chosen, when possible, one year prior to the incumbent's scheduled retirement.
4. Associate editors should be appointed by the editor-in-chief and should sit with the Editorial Committee at all regular meetings.

One of the first problems confronting Dr. Ferris and his associates, Drs. I. Arthur Mirsky and William B. Bean, was that of modernizing the Journal's business operations. During its six year sojourn in Boston, the Journal had grown from a barely self-supporting enterprise to a substantial business undertaking. It soon became apparent to the new editors that the bookkeeping methods and facilities which had been appropriate for an earlier period were inadequate for 1947. Accordingly, they hired a certified public accountant and lawyer as a consultant and, with his help, the Journal received a financial face-lifting. Its affairs were set in order, the real value of its accounts and property determined, and a modern, double-entry bookkeeping system instituted. In his 1948 report to the Council, Dr. Ferris expressed the opinion that the time had come when the services of a professional business manager were required.

The steady rise in circulation which had begun during the war continued—between 1946 and 1951, the number of subscribers doubled reaching nearly 4,000 by the end of Dr. Ferris' term. The small advertising income which had resulted from Dr. West's campaign in 1940 had been successfully encouraged by Dr. Gamble during the first years of his term. Even before he assumed office, Dr. Ferris had begun casting about among

the drug houses of the country for additional advertisers. It was his hope that he might induce a select group of firms to commit themselves to taking space in each issue for a period of from three to five years. To this end, he prepared a letter to potential advertisers and, along with it, a prospectus of the Journal in which he emphasized the fact that its influence was far more extensive than its circulation.

As a result of this campaign, which Dr. Ferris described in his 1949 report as "moderately intense," income from advertising rose during 1948 by about one-third over the previous year. In 1949, the editors stepped up their efforts to a high pitch with an "extensive" campaign, but response was unimpressive. Despite Dr. Ferris' arguments, the Journal's low circulation figure was still regarded as the major deterrent. Consequently, he set out to *prove* that "the size of the subscription list is not an adequate index of the potential market represented by the subscribers." To this end, a survey was conducted among 2,627 Journal readers designed to elicit detailed information regarding the amount of money they were instrumental in spending for apparatus, chemicals, biologicals, drugs and books. On the basis of a 29 per cent response, which Dr. Ferris believed to be a "fairly representative sample," he estimated that Journal subscribers were responsible for the expenditure of approximately $50,000,000 annually (although it should be noted that the survey did not eliminate the possibility of duplication due to overlapping responsibility). Potential advertisers were once again circularized and income from advertising began to show a gradual gain which was considerably enhanced when, in January, 1950, the Journal began publication on a monthly basis.

Dr. Stanley E. Bradley succeeded Dr. Ferris as editor-in-chief in mid-1952. Under Dr. Bradley, and under the present editor, Dr. Philip K. Bondy, there have been few major changes in Journal policies and operations. This fact is perhaps a tribute to the success with which Dr. Ferris and his associates had guided the Journal through its transition from an amateur undertaking to a large-scale enterprise conducted on an efficient, professional level. The streamlining of operations envisioned in the 1947 reorganization plans was, in large measure, realized during the Journal's five years in Cincinnati. The story of its development since then can be told almost entirely in statistical terms: a continuing growth of the subscription list; a slowly rising income barely keeping pace with rising production costs; a substantial gain in the number of manuscripts submitted each year and a concomitant growth in the average size of volumes.

The latter point is deserving of special mention. Following the wartime slump experienced after 1943, papers began to flow into the editorial office in greater and greater numbers. During Dr. Bradley's first full year as editor (1953) the total reached an all time high of 336, an increase of approximately 30 per cent over 1952. Although this was an unusually large jump, the upward trend has continued—in 1958 a total of 417 papers was submitted and a new editorial problem created thereby.

Over the past eight years, the proportion of manuscripts considered suitable for publication, with or without revision, has remained approximately constant. All indications are that the total number of incoming papers will continue to increase each year. If the percentage of those eventually accepted fails to change, it may become necessary once again to take considerations of space into account in making editorial decisions. This is not really a new problem, but an official policy of rejecting acceptable material for lack of space would be a new solution.

During many of its insecure years, Journal editors were forced to limit contents in accordance with available funds. As far as is known, however, they accepted papers on the basis of merit alone, maintaining a backlog to be published whenever finances permitted rather than turning worthy material down. Furthermore, they endeavored to keep the size of each issue fairly uniform, presumably in order to spread expenditures evenly over the year.

Since 1947, the size of the Journal has expanded and contracted primarily as a function of the amount of available material, but the ever higher costs of publication have kept pace with gains in total income and, in some years, have outstripped them. As Dr. Ferris pointed out, the Journal was caught in the rather anomalous situa-

tion of successful expansion resulting in decreased net income. Although its assets were considerable, the editors deemed it inadvisable to utilize reserves for current expenses and for this reason, obtained authority to raise subscription rates for the year 1951.

The same cycle has repeated itself since then. In his 1954 report, Dr. Bradley stated that a backlog was not maintained, manuscripts being sent to the press when they were accepted and the size of each issue varying accordingly. During the first three years of his term, the Journal operated at a loss and, in 1957, subscription rates were once again elevated. This provided temporary relief, but as Dr. Bondy pointed out in his report this year:

> Our popularity raises a considerable financial problem. The Journal has always accepted for publication whatever manuscripts were considered suitable by the Editorial Board without permitting considerations of available space to enter into the decision. We have also attempted to present all of the evidence, even when this meant the publication of expensive tables and figures. If this desirable attitude is to continue, it is clear that the Journal will find it increasingly difficult to remain solvent.

Perhaps a historical perspective offers some solace: although the difficulties of remaining solvent have a very familiar ring, the experience of the past indicates that Journal editors are skillful in finding ways to surmount them.

The "administrative" history of the Journal, thus brought up to date, is only a part of its story, standing as background to the 37 completed volumes which contain the lasting results of its 35 years of publication. Unlike many scientific organs, the Journal has confined its textual content to matters of strict scientific substance. Such journalistic sidelights as editorials, letters to the editors, book reviews, and review articles have been virtually absent from its pages. There are a few exceptions—Dr. Cohn's "Introduction" in the first issue, two obituaries (Dr. Francis Peabody in 1927 and Dr. Ferris in 1958), Dr. Austin's history of the Society published in 1949—but these are rare. Even the addresses of Society presidents were excluded until 1938, and since then an occasional president has refused to submit his remarks on the grounds that they consume precious space which might better be used for the dissemination of scientific information. Perhaps the most notable departure from this philosophy was that displayed by Dr. Ferris with the introduction of a feature entitled "Letter from the Editors." Its insertion into the Journal's pages was, as might be expected, a rather controversial matter and the feature was discontinued by Dr. Bradley when he succeeded to the editorship in 1952.

Primarily, the "Letters" were designed to answer frequently recurring questions regarding Journal operations or policies. One, for example, described the manner in which manuscripts were processed in an effort to explain why *some* delay between submission and publication was unavoidable (4). Another (5) discussed plagiarism in scientific writing, noting the occasional paper received by the editors which contained large sections lifted directly from the writing of another investigator, and reminding contributors of their obligation to give credit where credit was due.

One of the most interesting of the letters (6) dealt with another frequent question: "What is and what is not clinical investigation?" The Editors approached their answer by describing some of the stages through which clinical investigation had been carried on. Changes in the focus of interest among investigators, such as those outlined in the "Letter," are more fully documented by the steady succession of reports from clinic and laboratory which has found its way into the pages of the Journal during its 35 years of publication. An analysis of its scientific contents, thus, reveals many of the varying answers which have been given to the question, "What is and what is not clinical investigation?"

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HISTORY OF THE JOURNAL OF CLINICAL INVESTIGATION, 1924-1959

II. SCIENTIFIC CONTENTS

By PHILIP K. BONDY, M.D.

Editor-in-Chief, Journal of Clinical Investigation

The most interesting aspects of the history of the JOURNAL OF CLINICAL INVESTIGATION are those pertaining to its contents. Since the editors have, from the beginning, printed unsolicited manuscripts, the subjects of the papers which have been published have depended to some extent on the interests and attitudes of its authors. One might speculate at length as to what passes through an author's mind when he decides where to send his manuscript. Certainly, such considerations as the nature of the article, the audience for which it is intended, the probability of its acceptance, and the prestige of the journal as compared with others in the field enter into his decision. Undoubtedly all of these factors have influenced the material available to the editors of the Journal. The interests and critical abilities of the editors have, in turn, modified the selection of the articles which have finally been accepted. In order to evaluate the pattern which has resulted, I have reviewed briefly the contents of the Journal over the past 35 years. I have paid particular attention to evidence of changing interests of the contributors and to the introduction of new attitudes and techniques into the field of clinical investigation, as mirrored by the articles published by the Journal.

One of the most clear-cut results of this survey is the unexpected fact that little change has occurred through the years in the distribution of articles among most of the major fields of interest. Within fairly narrow limits, the percentage of papers dealing with problems in gastroenterology, infectious diseases or the processes of metabolism has remained unchanged. There has, however, been a gradual reduction in the proportion of papers dealing with the circulatory system. Since "competing" specialty journals in all of these fields began publication after the establishment of the Journal, it appears that the availability of other channels for diffusion of scientific knowledge has had a variable effect on what is submitted to the Journal for publication.

The major interests of our authors have, of course, changed with the years, but certain problems appear to have exerted a continuing fascination on clinical investigators. Throughout its 35 years, the Journal has carried papers concerned with such topics as the measurement of body fluid compartments, the factors regulating the balance of electrolytes, the mechanism of edema and ascites formation, the nature of "shock" and factors which determine its reversibility, the cause of arterial hypertension, and the measurement and clinical significance of a variety of pulmonary functions. Although the methods which have been brought to bear on these subjects have evolved greatly, satisfactory answers to certain of the problems have still not been obtained. In other cases (such as those concerned with body fluid compartments) the determination of acceptable normal values has been followed by investigation of clinical and physiological situations of increasing complexity. The introduction of new techniques has usually called for re-investigation of previously determined data. For example, plasma volume was first investigated by the dyes "brilliant vital red" and later with "Evans blue" (T-1824), introduced into clinical investigation by Gibson and Evans, J. clin. Invest., 1937, **16**, 301. The results obtained by these methods were later confirmed by measuring the distribution of I^{131}-labeled human serum albumin (Crispell, Porter and Nieset, 1950, **29**, 513) and by the use of radioiron or chromium-labeled erythrocytes (Sterling and Gray, 1950, **29**, 1614) combined with the hematocrit.

Although these general topics have continued to occupy the attention of our authors, the degree of interest in the different problems has varied as techniques have changed, and as external influ-

ences have provided impetus for special "practical" studies. The investigation of shock, for example, was greatly expanded during the Second World War and the Korean War. The intensive study of agents to protect against arsenical poison gases resulted in the publication of a mass of material related to "British Anti-Lewisite," which appeared as a supplement to the Journal. The wartime interest in blood preservation and the therapeutic use of plasma, either in its native form or after fractionation, resulted in a flood of papers related to these subjects. Our authors have always been interested in the study of infectious diseases, but the mobilization of large numbers of soldiers and their maintenance in tropical regions sparked a particularly intense study of such diseases as infectious hepatitis, malaria and influenza. Although some of these manuscripts were gathered into special supplements, many appeared separately in the general pages of the Journal during the later part of the war and immediately thereafter.

Certain other topics have apparently been of interest for only restricted periods of time. If one divides the past life of the Journal into three parts, beginning in 1924, 1938 and 1948, it becomes apparent that certain issues which occupied the energies of investigators during the first period had ceased to interest them in later periods. Studies of the electrocardiogram, for example, have occupied only a very small amount of space in our pages during recent years, although when the Journal first began publication, many papers were concerned with the mechanisms and interpretation of cardiac electrical phenomena. A great deal of attention was paid to the measurement and evaluation of the concentrations of plasma electrolytes and proteins in the early years. The pioneering work (Volumes 1–11) of J. P. Peters, F. W. Sunderman, J. H. Austin, and their colleagues in adults, and of A. F. Hartmann and D. C. Darrow in children, applied scientific principles to the study of the changes of body composition in disease states, and laid the foundation for modern clinical chemistry. The application of similar techniques by Atchley, Loeb, Richards, Benedict and Driscoll (1933, **12**, 297) to the study of diabetic acidosis underlies all modern concepts in the treatment of this disease. Laboratory methods introduced by these and other workers have proved so successful in the clinic that they have tended somewhat to supplant the Oslerian tradition of bedside diagnosis. The gradual progression of these techniques from the experimental laboratory to the routine care of patients represents a triumph of the principles to which the Society and the Journal are dedicated. But, as these methods met with widespread acceptance, they have ceased to be of much interest to those in the forefront of medical research. Very little material related to the concentration of plasma electrolytes and proteins has appeared in the pages of the Journal during the second two periods of its existence. Similar remarks might be made regarding the evaluation of tests of gastric analysis or the mechanism of action of the anti-anemic fraction of liver extract. The latter returned to a position of interest, however, when the isolation and characterization of vitamin B_{12} provided an opportunity for the specific studies of utilization and transport which have appeared in recent years.

The study of renal physiology has pursued an interesting pattern. During the early years, the study of urine morphology and the total quantities of various urinary constituents occupied most of the interest of our authors. With the introduction of the urea clearance technique, interest in renal function began to assume a somewhat more modern aspect. It was not until the second period of the Journal's life, however, that the introduction of specific renal clearance techniques such as the inulin clearance (Shannon and Smith, 1935, **14**, 393), diodrast clearance and para-amino hippurate clearance (Smith, Finkelstein, Aliminosa, Crawford, Graber, Goldring, Chasis, Ranges and Redish, 1945, **24**, 388 and 583) inaugurated the flowering of renal physiological investigation which continues to the present.

From 1938 to 1948 new problems occupied our authors. During this time, the ballistocardiograph was studied intensively. The relation of the kidney to hypertension was investigated by elucidation of the renin system. The relationship between streptococcal disease and rheumatic fever was elaborated. New methods permitted a study of the nature of the plasma lipids and their change in disease. The sulfonamides, followed by penicillin and streptomycin, came in for increasing atten-

tion. The pattern of medical science and clinical practice was changing so rapidly during this period that often by the time studies were published they were somewhat outdated. For example, the highly informative paper by Wood (1940, **19**, 95) on the clinical estimation of antiserum titer in patients with pneumonia appeared after the development of sulfonamides and penicillin had begun to render interest in antipneumococcal serum of only theoretical interest.

The recent interest in the application of radioactive substances to the measurement of physiological processes was foreshadowed by the experiments of Blumgart and Weiss, who used radium-C to measure the circulation time (1927, **4**, 15). Aside from their series of experiments, which lasted until 1930, however, radioactive measurements were not used until the late 1930's. The real spurt of interest in the techniques made possible by these substances, however, belongs to the postwar period. During the past 10 years, few fields of experimental medicine have escaped the influence of the counter or mass spectrometer. The availability of isotopic tracer substances has encouraged another recent development, namely the introduction of elaborate mathematical systems for the treatment of kinetic data which could be derived from such tracers. In leafing through the pages of the Journal, one is struck by the rarity with which mathematics was applied to the analysis of experimental data during the first two-thirds of the Journal's existence. Even the use of statistical analysis, a shibboleth of present scientific writing, did not appear until about the twelfth volume (1933) when Starr, Collins and Wood defined for their (presumably statistically naive) readers such parameters as the standard deviation, coefficient of correlation, slope of a regression line, and so forth. In the beginning, when mathematics was applied, it often took the form of a simple repetition of basic laws of physics, usually dealing with pressure, flow, viscosity and other parameters of the circulatory system. The introduction of differential equations to our pages has been a recent and (for some) traumatic experience. It is little solace to reflect that the mathematics commonly employed by our authors is usually of a variety which mathematicians find most pedestrian. It seems likely that the present trend toward the erection of mathematical models will expand, and that the days of simple arithmetical innocence are gone forever from our pages.

Experimental techniques have changed during the past 35 years, but not, perhaps, so basically as might have been expected. We still have today, as we did in the beginning, the metabolic balance study, the Fick Principle, and the epidemiological study. The application of these methods is somewhat different, however. Whereas, in earlier days, one studied the balance of sodium or nitrogen, one is now likely (in addition) to study the balance of steroids, amino acids, trace metals or vitamins. No longer does the cardiologist ventilate his subject with ethyl iodide; he transfixes him with a cardiac catheter instead. But he still applies the time-honored mathematics of Fick (published elsewhere in 1870). The fact that many of the early papers concerned with the development of the cardiac catheter technique were published in this Journal (Cournand, Riley, Richards and their co-workers, Volumes 24–28) probably accounts for the presence of papers which have expanded and extended the methods to include the study of congenital heart disease (Dexter, Haynes, Burwell, Eppinger, Seibel and Evans, 1946, **26**, 547), estimation of hepatic blood flow (Bradley, Ingelfinger, Bradley and Curry, 1945, **24**, 890), coronary blood flow and extraction of various substances by the kidney. But all of these extended catheterization methods still hark back to Fick.

The study of infectious disease, especially in its epidemiological aspects, has changed very little, but the weapons now used are virus isolations and complicated immunological techniques, whereas years ago bacterial cultures and fairly simple antibody tests were used.

As clinical researchers have become increasingly basic-science minded, they have tended to introduce an increasing interest in biochemistry and particularly in enzymology, specific chemical isolations and accurate fractionation procedures. An interest in enzymes has been present from the beginning, but originally it was the digestive enzymes which were studied. Later, the presence of certain enzymes in the blood was related to disease processes. A case in point is the description, by Gutman and Gutman (1938, **17**, 473), of the ele-

vation of serum acid phosphatase in patients with metastasizing prostatic cancer. Other enzymes have entered the picture until at present the study of plasma enzymes is, in itself, an overwhelming preoccupation of a certain part of the medical community. The Journal has recently published a number of papers concerned with this phase of clinical research. But more basic has been the increasing number of papers which have applied the disciplines of enzymology to the explanation of disease processes. For example, we have recently published several papers concerned with the relationship of the anaerobic metabolism of carbohydrates in erythrocytes to hemolytic disease.

The interest in purification and fractionation of complex biological substances is, perhaps, best exemplified by the extensive studies concerned with the separation of the various protein fractions of plasma. Most of the papers emanating from the laboratory of Cohn and his successors have appeared in the Journal; indeed, an entire supplement was devoted to them in 1944. The expansion of this type of study to provide information about the protein-binding of trace substances such as iron and copper, and to supply useful products such as antihemophilic globulin, gamma globulin, fibrin, fibrinogen and albumin, is a story which has been written, for the most part, in the pages of the Journal.

Considering the fact that the Journal was established specificially to publish the results of investigations of clinical problems, it is not surprising that most of the material published in its pages can be related in some way to disease processes or to the establishment of normal values against which impaired function may be measured. There has, however, been a notable paucity of papers concerned with the presentation of clinical observations based on bedside observation. Moreover, such observations have tended to become even rarer as the years have progressed. In spite of this, purely clinical papers continue to appear occasionally in the pages of the Journal, usually in conjunction with a related experimental or epidemiological study. The plaintive comments of some of our readers that "there is nothing very clinical about the JCI any more" are not supported by the facts. Many of the studies reported in these pages have originated in attempts to answer clinical questions. The continuous progress of clinical medicine has been based on apparently "impractical" scientific studies of disease.

From the very beginning, the editors have attempted to publish only "worthwhile" papers. Since mere repetition of previous studies is not likely to be rewarding, most of the material in the Journal represents a "first" (or at least a fairly close "second") of some sort or other. It is undoubtedly brash, therefore, for me to attempt to select from our archives specific papers which seem particularly important. In spite of this, I have been so impressed by the impact of a few of the papers on present scientific or clinical thought and practice that I could not refrain from singling out one or two for particular mention. I have already cited some of them. Others, which were published within the past 10 years or so, are so recent that it seems too early to single them out. But among the early papers were several more which seem worth mentioning.

The introduction of a new viewpoint may be of great importance. The diagram of the plasma ionic pattern in a paper by Gamble and Ross on page 419 of Volume I must represent one of the very earliest uses of what later generations of medical students were to know as the "Gamblegram."

The fact that parathyroid hormone exerts a primary effect on the renal excretion of phosphorus was described by Albright, Bauer, Ropes and Aub (1929, **7**, 139). The subsequent studies of calcium metabolism and parathyroid function by these and other authors resulted in a remarkable piece of scientific investigation and cross-country cooperation when a pair of papers were published by Hannon, Shorr, McClellan and Dubois (1929, **8**, 215) and Bauer, Albright and Aub (1929, **8**, 229) describing the same patient, Captain Charles Martel, who represented the first case of hyperparathyroidism diagnosed in the United States.

The "iron lung" is a familiar (and, we hope vanishing) piece of apparatus in most hospitals. It first saw light in the laboratories of Dr. Philip Drinker, and was launched in the Journal (Drinker and Shaw, 1929, **7**, 229). The prolonged studies of acidosis in children carried out in Hartmann's laboratory culminated, in a prac-

tical sense, in the introduction of the use of sodium lactate for the treatment of acidosis in children by Hartmann and Senn (1932, **11**, 327).

If these selections seem biased and arbitrary, it must be remembered that they are personal. Other reviewers would doubtless select other papers, for there is a huge store of information in the past issues of the Journal. A large part of this information is still as fresh and valid as it was years ago when it first was published. This continuing value arises from the high standards set by the editorial committees in conformity with the objectives of the Society. The Journal has been one of the most important vehicles for the expression of the Society's ideals, and (together with the annual meetings) has kept constantly alive the principles of excellence which impelled the founding fathers to form the Society. The struggle to maintain high standards has not always been easy, and the fact that they have been sustained is a tribute to the authors, reviewers and editors who have labored to bring this about. If your present editor ends this brief note with a feeling of pride it is for the traditions of the institution which he serves, rather than for any personal feeling of accomplishment. And this pride can be shared, not merely by members of the Society, but by all who have contributed to the progress of medicine in the past years, in whatever capacity they may have functioned.

ROSTER OF EDITORIAL STAFFS OF THE JOURNAL OF CLINICAL INVESTIGATION FROM 1924 TO THE PRESENT TIME

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* Normally, in May of that year. The date in parentheses following each name is the year of retirement.

† The Editorial Board varied in size from six to nine members. The entire Board was re-elected every two years by the Editorial Committee, with the provision that no more than two incumbents could be replaced at each election. Thus, members normally served several successive two year terms.

‡ The Editorial Committee (until 1948) normally consisted of 15 members elected for six year terms. After 1948, members served five year terms, with three members normally being elected each year. Names are grouped by year of election and arranged, from left to right, in order of the duration of service. § Assistant editor. ‖ Journal treasurer. ¶ Associate editor.